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In vivo Phosphorylation Sites of Barley Tonoplast Proteins identified by a Phosphoproteomic Approach

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Abbreviations
SCX - strong cation exchange, TEM - tonoplast enriched microsomes

Keywords
transport, vacuole, cation exchanger, channel, proteomics

Abstract

In plants the vacuole functions are the cellular storage of soluble carbohydrates, organic acids, inorganic ions and toxic compounds. Transporters and channels located in the vacuolar membrane, the tonoplast, are modulated by posttranslational modifications to facilitate the optimal functioning of a large number of metabolic pathways. Here we present a phosphoproteomic approach for the identification of *in vivo* phosphorylation sites of tonoplast (vacuolar membrane) proteins. Highly purified tonoplast and tonoplast enriched microsomes were isolated from photosynthetically induced barley (*Hordeum vulgare)* mesophyll protoplasts. Phosphopeptides were enriched by strong cation exchange chromatography (SCX) followed either by IMAC or titanium dioxide (TiO_2) affinity chromatography and were subsequently analyzed using LC-ESI-MS/MS. In total, 65 phosphopeptides of 27 known vacuolar membrane proteins were identified, including the two vacuolar proton pumps, aquaporins, CAX transporters, Na^+/H^+ antiporters as well as other known vacuolar transporters mediating the transfer of potassium, sugars, sulphate, and malate. The present study provides a novel source to further analyze the regulation of tonoplast proteins by protein phosphorylations, especially as most of the identified phosphorylation sites are highly conserved between *Hordeum vulgare* (*Hv*) and *Arabidopsis thaliana*.

Introduction

In mature plant cells the vacuole is by far the largest organelle and occupies up to 90% of the cell volume. The vacuole serves as a temporary store for a large number of metabolites and ions, such as sugars, organic acids, amino acids, potassium, phosphate and nitrate just to mention a few. The identification and characterization of vacuolar membrane proteins was much slower compared to the plasma membrane located proteins, mostly since no appropriate yeast mutants were available allowing complementation studies. However, in the last few years considerable progress has been made and a large number of vacuolar membrane (tonoplast) proteins have been identified and characterized [1]. This progress was due to the complete sequencing of the Arabidopsis genome in 2000, the comparison of Arabidopsis with already known sequences and the generation of knock-out lines for nearly all Arabidopsis genes.

In addition to the molecular identification and characterization of specific transporters and channels, several vacuolar proteomic approaches have been performed. Such an approach has been undertaken for Arabidopsis, cauliflower and barley [1-8]. Between 34 and 650 different proteins, including soluble and membrane proteins were detected. From these proteins 23 to 195 were reported to be membrane proteins. This discrepancy can be attributed to different amounts of proteins isolated, and detection techniques used in the respective approaches. Interestingly, the overlap between identified proteins is relatively low. Besides tonoplast proteins, non-vacuolar proteins from other organelles such as mitochondria, plasma membrane and chloroplasts were additionally detected. The proportion of contaminants differed considerably between the different studies. Therefore, for all newly identified putative vacuolar proteins final proof for vacuolar localization has to be shown. However, this aspect has only been addressed in three of these studies [3,4,6]. Furthermore, the studies with cauliflower and barley showed that it is important to analyze different plant species and tissues. An alternative strategy to identify membrane proteins from organelles has been presented by Dunkley et al. [9]. Using density gradients in combination with isotope tagging they could differentiate membrane proteins from different organelles.

In plants, phosphoproteomic approaches of plasma membrane proteins have been reported the identification of 67 to 1127 phosphopeptides after elicitation and sucrose supply [10-12]. Recently, Prak et al. [13] analysed in particular the role of phosphorylation at the C-terminal tail of plasma membrane aquaporins in Arabidopsis roots and presented evidence that phosphorylation affect targeting of aquaporins.

Identification and characterization of the substrate specificity is not sufficient to understand the respective function of a vacuolar transporter or channel. The vacuole plays a role as a buffer and is the organelle allowing the plant to maintain a cytosolic homeostasis and an optimal functioning of the metabolic pathways occurring in the cytosol. For instance non-aqueous fractionation and *in vivo* NMR studies have independently shown that cytosolic malate concentrations are kept very constant, while vacuolar malate contents fluctuate diurnally or in response to environmental changes [14,15]. Changes in solute uptake and release activity can be obtained by altered expression. Nevertheless, in many cases a fast response is required and such a response is mostly mediated by protein phosphorylation/dephosphorylation.

There is increasing evidence that 14-3-3 proteins are regulating the activity of the vacuolar potassium channel KCO1 [16,17] as well as the activity of the vacuolar ATPase [18]. This regulation is mediated by the binding of the 14-3-3 proteins to the phosphorylated transporter or channel. Whiteman et al. [19] just published the first study on *in vivo* phosphorylation sites of Oryza sativa tonoplast proteins. They could identify 11 phosphorylated proteins (14 different phosphopeptides), which have been annotated as shoot and root tonoplast located, respectively. Here, we present a dedicated approach for phosphopeptide enrichment form purified tonoplast and tonoplast enriched microsomes from *Hordeum vulgare* (Hv), which allowed the identification of 65 phosphopeptides of known tonoplast proteins. The coverage of phosphopeptides was increased by (1) the use of two different enrichment techniques, namely IMAC and TiO_2, (2) the combination of strong cation exchange chromatography (SCX) with IMAC or TiO_2 affinity chromatography as well as (3) by the isolation of different starting materials; tonoplast enriched microsomes and purified tonoplast.

Materials and Methods

Plant Material

Hordeum vulgare var. Baraka seedlings were grown in soil for 8 days in a controlled environment chamber (16 hr light/8 hr dark, 300 $\mu Em^{-2}s^{-1}$, 22°C, 60% relative humidity).

Tonoplast Isolation

Barley mesophyll vacuoles were isolated from 8 d-old plants according to Rentsch and Martinoia [20], omitting BSA in all buffers. To induce photosynthesis before vacuole isolation, purified protoplasts were incubated in the presence of 15 mM $NaHCO_3$ and illuminated for 15 min. After disruption of protoplasts by forcing them through a syringe, 25 mM NaF was added to all buffers. Isolated vacuoles were stored in betaine buffer containing 10 nM okadaic acid. Tonoplast vesicles were collected by sonication of vacuoles followed by ultracentrifugation (1 hr, 100,000 x g). Vacuolar membranes were saline washed (0.3 M KI, 20 mM HEPES/KOH pH7.2, 25 mM NaF, 10 nM okadaic acid) by 15 min incubation on ice with occasional vortexing and subsequent ultracentrifugation for 45 min at 100,000 x g.

Before tryptic digest, 1 mg of KI-washed tonoplast proteins were resuspended in homogenisation buffer (100 mM HEPES/KOH pH 7.2, 25 mM NaF, 5 mM EDTA, 10 mM EGTA, 5% glycerol, 0.5% polyvinylpyrrolidone K30, 3 mM DTT, 1 mM PMSF, 50 mM sodium pyrophosphate, 1 mM sodium molybdate, 1 tablet protease inhibitors [Roche]) and an equal volume of Tris-buffered Phenol was added. After vortexting and 10 min centrifugation (10,000 x g) at 4°C the supernatant was discarded and an equal volume of back extraction buffer (100 mM Tris pH 8.4, 20 mM KCl, 10 mM EDTA, 0.4% β-Mercaptoethanol) was added. After vortexting and centrifugation (10 min, 10,000 x g) at 4°C, the supernatant was discarded and proteins were precipitated by adding the 5 x volume of 100 mM ammonium acetate in methanol. The protein pellet was washed once with 100 mM ammonium acetate in methanol and twice with 80% acetone.

Isolation of Tonoplast Enriched Microsomes

The epidermis of barley leaves was peeled and leaves were incubated in digestion buffer (0.5 M sorbitol, 1 mM $CaCl_2$, 10 mM MES pH 5.6) containing 0.7% Cellulase YC and 0.05% Pectolyase Y23 (both from Kyowa Chemical Products Co., Ltd., Tokyo, Japan) for 1.5 hr at 30°C. After digestion, protoplasts were recovered on a percoll cushion by centrifugation (1,500 x g for 10 min) and resuspended in buffer M

(250 mM sucrose, 100 mM HEPES/KOH pH 7.2, 25 mM NaF, 10 mM EDTA, 5% glycerol, 0.5% polyvinylpyrrolidone K30, 3 mM DTT, 1 mM PMSF, 50 mM sodium pyrophosphate, 1 mM sodium molybdate, 10 nM okadaic acid, 1 tablet protease inhibitors [Roche]). Protoplasts were ruptured by forcing them trough the needle of a syringe. In this lysate a large number of vacuoles were present. The lysate was centrifuged 4 x at 3,000 x g for 10 min. The pellet containing intact chloroplasts, mitochondria, peroxisomes and protoplasts was discarded and the supernatant was collected for ultracentrifugation (1hr, 100,000 x g). Harvested membranes were saline washed (0.3 M KI) as described (see tonoplast isolation). Before tryptic digest, 2 mg of tonoplast enriched membranes were desalted by acetone precipitation.

Isolation of Total Microsomes

Protoplasts were isolated as described above, centrifuged on a percoll cushion and resuspended in buffer A (50 mM HEPES/KOH pH 7.2, 10 mM EDTA, 5% glycerol, 0.5% polyvinylpyrrolidone K30, 3 mM DTT, 1 mM PMSF, 50 mM sodium pyrophosphate, 1 mM sodium molybdate, 1 tablet protease inhibitors [Roche]). Protoplasts were disrupted by sonication and centrifuged (3,000 x g, 15 min). The supernatant was ultracentrifuged for 1 hr at 100,000 x g to yield the total microsomal fraction. Total membranes were saline washed as described.

In-Solution Digestion with Trypsin

Protein pellets were resuspended in 300 µl of 25 mM NH_4HCO_3 and heated for 10 min at 90°C. Trypsin (Promega, Switzerland) was added (1µg trypsin per 50 µg protein) for overnight digestion at 35°C. Peptides were reduced by adding 10 mM TCEP and were incubated at 35°C for 30 min. The peptide containing solution was centrifuged for 10 min at 10,000 x g to remove insoluble material and peptides were dried in a speed vac.

Fractionation of Peptides by Strong Cation Exchange Chromatography

Peptides were dissolved in buffer A (10 mM KH_2PO_4 pH 2.6 in 25% acetonitrile) and loaded onto a 4.6x200 mm (fraction of tonoplast enriched microsomes) or 2.1x200mm (tonoplast fraction) polySULFOETHYL aspartamide A column (PolyLC, USA) on an Agilent HP1100 binary HPLC system. Peptides were eluted with an

increasing KCl gradient (10-40 min 0-30% buffer B, 40-60 min 30-100% buffer B, buffer B: 10 mM KH_2PO_4 pH 2.6, 350 mM KCl in 25% acetonitrile)
Five (tonoplast) or six (tonoplast enriched microsomes) fractions were pooled and desalted with Sep-Pak reverse-phase cartridges (Waters, UK).

Immobilized Metal Ion Affinity Chromatography (IMAC)

Chelating Sepharose Fast Flow beads (GE Healthcare) were charged four times with 0.1 M $FeCl_3$ and washed four times with washing buffer (74:25:1 water:acetonitrile:acetic acid). Desalted peptides were acidified with 0.1% TFA in 25% acetonitrile, applied to 40 µl of 25% bead slurry and incubated for 30 min at room temperature. Samples were washed five times with washing buffer and once with water. Phosphopeptides were eluted by adding 30 µl of 100 mM sodium phosphate buffer pH 8.9. The pH of all samples was adjusted to 3 with 10% TFA followed by desalting and concentrating samples using ZipTips (Millipore).

Titanium Dioxide (TiO_2) Affinity Chromatography

Phosphopeptides were enriched using TiO_2 affinity chromatography as described by Bodenmiller et al. [21] with minor modifications. Peptides were desalted and dissolved in a solution containing 80% ACN, 2.5% TFA and 0.13 M phthalic acid. The peptide mixture was incubated with 0.3 mg TiO_2 (GL Science, Saitama, Japan) for 30 min in blocked mobicol spin columns. After washing two times with 0.3 M phthalic acid solution in 80% ACN, two times with 80% ACN, 0.1% TFA and finally two times with 0.1% TFA, peptides were eluted with a 0.3 M NH_4OH solution and dried in a speed vac.

Analysis by LC-ESI-MS/MS

Samples were resuspended in 5% ACN, 0.1% formic acid and analyzed on a LTQ-Orbitrap mass spectrometer (ThermoFischer Scientific, Bremen, Germany) interfaced with a nanoelectrospray ion source. Peptides were separated using an Eksigent nano LC system (Eksigent Technologies, Dublin, CA, USA), equipped with a 11 cm fused silica emitter, 75 µm inner diameter (BGB Analytik, Böckten, Switzerland), packed in-house with a Magic C18 AQ 3 µm resin (Michrom BioResources, Auburn, CA, USA). In house pulling of the capillary allowed for spraying the eluted peptides directly from the tip of the column. Peptides were loaded from a cooled (10°C) Spark Holland auto

sampler and separated using ACN/water solvent system containing 0.1% formic acid with a flow rate of 200 nl/min. Peptide mixtures were separated with a gradient from 3 to 35% ACN in 90 minutes.

Up to 5 data dependent MS/MS spectra were acquired in the linear ion trap for each FT-MS spectral acquisition range, the latter acquired at 60,000 FWHM nominal resolution settings with an overall cycle time of approximately 1 second. Dynamic exclusion was switched on entailing that up to 500 m/z ± 20 ppm values were excluded from tandem MS for 120 second. For injection control the automatic gain control was set to 5e5 and 1e4 for full FTMS and linear ion trap MS^2, respectively. The instrument was calibrated externally according to manufacturers instructions. The samples were acquired using internal lock mass calibration on m/z 429.088735 and 445.120025.

Data Analysis

MS and MS/MS data were searched using Mascot version 2.1.04 (Matrix Science, London, UK). Database searching of MS/MS spectra was performed using a database, including the NCBI protein database of the Liliopsida (GI4447, 139,384 protein sequences) and barley EST consensus sequences. EST consensus sequences that are available at http://pgrc.ipk-gatersleben.de/cr-est/files/barley/ (consensus_g02; 14,151 consensus EST sequences) were translated into protein sequences using the OrfPredictor (https://fungalgenome.concordia.ca/tools/OrfPredictor.html) [22]. EST consensus sequences were BLAST-searched against the UniProt non-redundant protein sequences to identify the closest homolog.

Data were searched against the database restricted to tryptic peptides, missing maximal two cleavage sites, allowing for 2+ and 3+ charged peptides a parent mass error tolerance of 5 ppm and a daughter ion error tolerance of 0.8 Da. Modifications used include oxidation (M, variable) and phosphorylation (STY, variable).

Phosphopeptide identifications were accepted with a minimal Mascot™ ion score of 25 and a Mascot™ expect value of ≤ 0.05 [23]. A normalized delta ions-score (ΔI) was calculated of all phosphopeptides containing more then one serine, threonine or tyrosine residue by taking the difference of the two top ranking phosphopeptide ion scores and dividing that difference by the ion score of the first ranking phosphopeptide. Phosphorylation site assignments with a $\Delta I \geq 0.4$ were automatically accepted [24]. Phosphopeptides of tonoplast proteins were further manually

evaluated by verifying the neutral loss of H_3PO_4 (-98 Da). Neutral loss of H_2O and NH_3 were taken into account. Furthermore, a series of three y or b ions was required to accepted phosphopeptide identification of tonoplast proteins. Phosphopeptides holding a normalized delta ions-score of less than 0.4 [24] were manually inspected to assign the phosphorylation site within the phosphopeptide. Neutral loss events from b- and y-type fragment ions were utilized to determine the phosphorylation site within the peptide sequence. Ambiguous assignments of phosphorylation sites are indicated in Table 1.

Some phosphopeptide identifications are based on protein sequences of *Oryza sativa*. The corresponding cDNA sequences from rice were blasted against the barley IPK crop EST database (pgrc.ipk-gatersleben.de/cr-est/index.php) and the barley EST database of the Computational Biology and Functional Genomics Lab (compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=barley) to check if peptide sequences of rice and barley are different to exclude false phosphopeptide identifications.

Western Blotting

Membrane proteins (15 µg) were separated by SDS-PAGE using a 10% polyacrylamide gel. Proteins were electroblotted onto a PVDF membrane (Roche, Basel, Switzerland). Western blotting was carried out using antibodies against γ-tonoplast intrinsic protein (OsTIP2.1), plasma membrane intrinsic protein (OsPIP2.3), photosystem I light harvesting complex subunit 2 (LHCa2, Agrisera, Vännäs, Sweden) and photosystem II light harvesting complex subunit 3 (LHCb3, Agrisera). Secondary antibodies, anti-rabbit coupled to alkaline phosphatase (AP), were diluted 1:3,000 in TBST (0.2 M Tris-HCl pH 7.5; 0.5 M NaCl; 0.05% TWEEN 20). AP detection mixture was prepared by adding 6.6 µl NBT (Promega) and 3.3 µl BCIP (Promega) to 1 ml AP buffer (0.1 M Tris-HCl pH 9.5; 0.5 M $MgCl_2$).

Results

For a successful phosphoproteomic approach the amount for starting material is crucial. We have previously shown that vacuoles isolated from the crop plant barley (*Hordeum vulgare*) are very suitable for proteomic studies, since large amounts of highly purified tonoplast can be obtained [3]. On the other hand it has to be considered that the barley genome is not fully sequenced. However, Blast searches

of identified barley tonoplast proteins show 80-90% identity to their rice homologues and especially regulatory sites of proteins, as phosphorylation sites, are expected to be highly conserved.

To search for phosphorylation sites of tonoplast proteins we isolated two different starting materials, highly purified vacuolar membranes as well as tonoplast enriched microsomes, from photosynthetically induced barley mesophyll protoplasts. Isolated membranes were treated with high salt concentrations (0.3 M KI) to reduce the amount of peripheral-bound proteins as we were primarily interested in phosphorylations of highly hydrophobic tonoplast transporters and channels.

Using Western blotting we compared the quantity of tonoplast, plasma membrane and thylakoid proteins between the purified tonoplast vesicles, tonoplast enriched microsomes and total microsomes. Western blotting experiments (Figure 1) demonstrate that the tonoplast protein γ -TIP (gamma-tonoplast intrinsic protein) is strongly enriched in the purified tonoplast fraction and of higher abundance in the tonoplast enriched microsomes compared to the total microsomes. Thylakoid proteins (LHC-a2, LHC-b3) are depleted in the tonoplast enriched microsomes compared to the total microsomes. The purity of tonoplast vesicles is shown by the absence of thylakoid proteins (LHCs) and the plasma membrane intrinsic protein (PIP).

Phosphopeptide Enrichment and Identification

We applied several approaches in parallel (Figure 2) to increase the coverage of identified phosphorylation sites of barley tonoplast proteins. Tonoplast enriched microsomes (1.8 mg) and highly purified tonoplasts (1 mg) were digested with trypsin and the resulting peptide mixtures were pre-fractionated using strong cation exchange chromatography (SCX), which separates phosphopeptides from the majority of non-phosphorylated peptides due to different net-charges [25]. Five (tonoplast) to six (tonoplast enriched microsomes) pooled SCX-fractions were desalted, split and phosphopeptides of each fraction were further enriched either by IMAC or titanium dioxide (TiO_2) affinity chromatography. In addition, tryptic peptides derived from tonoplast (200 µg) and tonoplast enriched microsomes fractions (200 µg) were split after desalting and incubated on IMAC or TiO_2 columns without previous SCX (Figure 2). After measurement by LC-ESI-MS/MS, database searches

were performed by Mascot using a database including the NCBI Liliopsida protein sequences and translated barley EST consensus sequences.

Several phosphopeptides were assigned to protein sequences of rice (*Oryza sativa*). To exclude false positive phosphopeptide identifications of tonoplast proteins, the corresponding cDNA sequences of the identified rice proteins were blasted against the IPK crop EST database (pgrc.ipk-gatersleben.de/cr-est/index.php) or against the barley EST database of the Computational Biology and Functional Genomics Lab (compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=barley). Phosphopeptide identifications based on rice sequences were only accepted if no amino acid exchange between the barley and rice peptide sequence was observed. Unfortunately, no barley EST sequence was available for the phosphopeptide KAPpSMEWR of the potassium transporter HAK15.

To validate the phosphorylation site assignment within a phosphopeptide with more than one serine, threonine or tyrosine a normalized delta ion score (ΔI) was calculated [24]. For phosphopeptides with a $\Delta I < 0.4$ the phosphorylation site assignment was manually inspected. Phosphorylation site assignments with a $\Delta I \geq 0.4$ were automatically accepted.

Overlap of Identified Phosphopeptides

As demonstrated by Bodenmiller et al. [21] the overlap of phosphopeptides isolated by different enrichment methods is relatively low. In the present study, most of the phosphopeptides were detected in more than one fraction. However, several phosphopeptides of tonoplast proteins could only be enriched either by IMAC (12 phosphorylation sites) or TiO_2 affinity chromatography (3 phosphorylation sites).

If tryptic peptides were pre-fractionated by SCX prior to further enrichment using IMAC or TiO_2 affinity chromatography, the number of identified phosphorylation sites were increased. Twenty-three phosphorylation sites of tonoplast proteins were detected using only IMAC and TiO_2 affinity chromatography and 39 phosphorylation sites of tonoplast proteins were identified using SCX prior to further enrichment (only one phosphorylation site was exclusively found when using only IMAC or TiO_2 affinity chromatography). This is congruent with a higher degree of phosphopeptide enrichment. The first two SCX fractions incubated either on IMAC or TiO_2 columns consisted primarily of phosphopeptides. In these first SCX fractions 75-90% of all detected peptides were phosphopeptides (see Supplementary Figure 1).

In addition, we tested two different starting materials, purified tonoplasts and tonoplast enriched microsomes. More than 400 phosphoproteins were detected in the tonoplast enriched microsomes and 53 phosphoproteins were identified in the tonoplast fraction. Surprisingly, more phosphorylation sites of tonoplast proteins were identified in the tonoplast enriched microsomes (37 phosphorylation sites) than in the purified tonoplast fraction (27 phosphorylation sites; Figure 3A). The organellar distribution of all detected phosphoproteins in the tonoplast enriched microsomes and the purified tonoplast fraction is indicated in Figure 3B-C. In the tonoplast enriched microsomes 6% of all phosphoproteins are known while in the purified tonoplast fraction this percentage is 43.5% and for 22.6% of all phosphoproteins the subcellular localization is unknown. Among the proteins of so far unknown localization tonoplast proteins might be present. Since the tonoplast contains less than 1% of the cellular protein, these results indicate that the vacular fraction is contaminated with less than 1% with proteins of other subcellular compartments. Nevertheless, a high purity of vacuolar membranes is not necessary to detect phosphopeptides of tonoplast proteins. One reason might be that dephosphorylation processes, even in the presence of phosphatase inhibitors, might be underestimated during the time-consuming isolation of tonoplast (25 µg per day) and the storage of the vacuolar membranes over a long period to collect the amount of tonoplast required. Moreover, the higher sample complexity of the tonoplast enriched microsomes is probably not a limiting factor, since it gets strongly reduced during the phosphopeptide enrichment procedure.

Phosphorylation Sites at Vacuolar Membrane Proteins of Barley

Using the parallel approach described above more than 600 unique phosphopeptides were identified in the fraction of tonoplast enriched microsomes including phosphopeptides from membrane and soluble proteins (Supplementary Table 1). Previously published phosphorylation sites at plasma membrane proteins were observed confirming our strategy of phosphopeptide enrichment and identification (e.g. ISAEDEMAGMDLpTR (T460) and HGGFAYVYHDDDEHDKpSVGGFMLR (S471) of the ammonium transporter AMT1, the C-terminal phosphopeptide GLDIDTINQNYpTV (T958) and pTLHGLQAPEPASHTLFNDK (T565) of the plasma membrane H^+-ATPase, C-terminal phosphopeptide ALGSFRpSNA (S276) of a plasma membrane intrinsic

protein). The SUBA database (http://www.suba.bcs.uwa.edu.au) [26] was used to clarify the subcellular localization of several membrane proteins.

In Table 1 all 65 phosphopeptides of tonoplast proteins detected in our study are listed (for spectra see Supplementary Figure 2). The proteins are classified to vacuolar transporters, ion channels, aquaporins and pumps. Within these 65 phosphopeptides were identified, 40 phosphorylation sites and 13 ambiguous phosphorylation sites assigned to 27 known tonoplast proteins. Of these 53 phosphorylation sites (40 unambiguous + 13 ambiguous phosphorylation sites) 73.5% were independently reproducible by the use of different enrichment strategies, starting materials or by differently cleaved peptides (none or one missed tryptic cleavage site). Serine was the major amino acid phosphorylated (50 phosphorylation sites, 93%), while three phosphorylated threonines (7%) were observed. Not a single phosphorylation site at the amino acid tyrosine was found. We believe that the chance to detect a phosphotyrosine in our study is generally low because the frequency ratio of phosphoserine:phosphothreonine:phosphotyrosine is in general 1800:200:1 [27]. As expected the protein phosphorylations were localized at the N- and C-termini or in intracellular loops of the vacuolar membrane proteins. The assignment of the phosphorylation sites to intracellular loops as well as to the C- and N-termini of the tonoplast proteins might also be an indication of their topology, since ATP, as the substrate of protein kinases, is not localized inside the vacuole [30].

Protein kinases recognize a specific site represented by the amino acids surrounding the residue to be phosphorylated. Six amino acids upstream and downstream of the 40 unambiguous phosphorylation sites were sorted and the relative frequency of the amino acids was determined to search for common motifs (Figure 4). Most amino acids were evenly distributed over all positions (see leucine/isoleucin and alanine). In contrast aromatic amino acids showed a preference for the position +1 and +6. For the basic amino acids arginine and lysine an overrepresentation was found at position +2 and +4 as well as a strong preference of histidine at position +2. Glycine was mainly found at position -1 and was underrepresented at position +1 and +2. Acidic amino acids were randomly distributed, except that at position +4 acidic amino acids were virtually absent. No particular dominance of proline at position +1 was found. These information implied at least a contribution of PKA-like (R,K-x-pS, R,K-x-x-pS) and PKC-like (pS-x-R,K) kinases in the phosphorylation of tonoplast proteins.

Discussion

Performing one of the first systematic approach to search for phosphorylation sites of tonoplast proteins 65 different phosphopeptides (Table 1) were detected, resulting in the identification of 40 phosphorylation sites and 13 ambiguous phosphorylation sites of 27 known tonoplast proteins. Only proteins have been considered, if experimental evidence for the tonoplast localization of the protein or a close homologue is available. With the exeption of the 116 kD subunit of the V-ATPase, which is organelle specific, all other subunits of this ATPase are also present in the Golgi and ER. In contrast, all other membrane proteins listed as tonoplast proteins have been exclusively localized in the tonoplast. The number of identified phosphopeptides was increased by the combination of different enrichment techniques and by the use of different starting materials, purified vacuolar membranes and tonoplast enriched microsomes (Figure 2 and Figure 3). We showed that the extensive isolation of tonoplasts with high purity is not mandatory to identify phosphorylation sites of its intrinsic proteins as more tonoplast specific phosphorylation sites were identified in the tonoplast enriched microsomes than in the purified tonoplast fraction (Figure 3). This conclusion surely depends strongly on the annotated protein localizations. Since the percentages of unknown proteins are very high in both fractions, the ratios of the amount of phosphoproteins located in the tonoplast and proteins located in a different cell compartment are most likely underestimated. The extent of underestimation might differ in the two fractions.

Based on the distribution of amino acids surrounding the residue to be phosphorylated (Figure 4) it can be proposed that PKA-like and PKC-like kinases are phosphorylating tonoplast proteins. This information will be useful to search for protein kinases involved in tonoplast protein phosphorylation.

In comparison to Arabidopsis plasma membrane phosphoproteomic studies a smaller number of phosphopeptides was detected by our approach. The first global analysis of the plasma membrane phosphoproteome identified 283 phosphopeptides [10]. In two different quantitative phosphoproteomic studies of the plasma membrane have been detected 67 [12] and 1172 phosphopeptides [11], respectively. However, the approach of Benschop and Mohammed et al. [11] included soluble proteins and only 25% of all identified phosphopeptides belonged to membrane proteins with more than two transmembrane domains. Although the inclusion of barley EST sequences into the searched protein database increased the number of identified proteins

substantially, a part of the phosphopeptides might be not attributed during the database searches due to the fact that the barley genome is not completely sequenced. This may also explain that no phosphorylation sites for multi drug resistance associated proteins (MRPs) were observed, since for these transporters only protein sequences from rice and partial EST sequences are available. Furthermore, it must be considered that the vacuolar membrane contains far less proteins than the plasma membrane and other internal membranes.

Previous publications have already described the regulation of vacuolar ion channels [16,17] and of the vacuolar ATPase [18] by 14-3-3 proteins, what can be confirmed now by our comprehensive tonoplast phosphoproteome analysis. The vacuolar transport of water, amino acids, protons and ions as sulphate, potassium, sodium, calcium and chloride seems to be modulated by protein phosphorylation/dephosphorylation of the respective transporters and channels.

Ten phosphopeptides were determined for the two major tonoplast pumps, the vacuolar H^+-ATPase and the vacuolar pyrophosphatase, suggesting a tight regulation of these proteins via posttranslational modifications. *In vitro* phosphorylation assays have been shown the phosphorylation of the B subunit of the vacuolar H^+-ATPase [29] and provided evidences that several subunits of the vacuolar H^+-ATPase are potential targets of WNK kinases [30]. Here, we observed phosphosites at five subunits of V_1-sector (A, B, C, D, and H) and one phosphorylation site at the subunit A of the V_0-sector (Table 1). These results should allow a deeper insight into the regulation and possible assembly of the vacuolar H^+-ATPase. Three phosphorylation sites were identified at the vacuolar pyrophosphatase, the proton pump, which plays a central role during the early stage of plant development [31].

We found two different vacuolar Na^+/H^+ antiporters (gi_28201131, gi_29825705) phosphorylated at the C-terminus (Table 1). A rapid induction of the vacuolar Na^+/H^+ exchange activity has been shown by the addition of Na^+ to barley roots [32]. This activation was also seen in the presence of protein synthesis inhibitors, suggesting that posttranslational modifications, such as phosphorylations, are involved in the induction of vacuolar Na^+/H^+ antiporters. The closest homologue of the vacuolar Na^+/H^+ antiporter (gi_29825705) in Arabidopsis is AtNHX1 (66% homology). A topology analysis of AtNHX1 in yeast revealed a luminal C-terminus [33], which interacts with calmodulin within the vacuole to regulate the cation selectivity of the transporter [35]. The identified phosphorylations at the C-terminus suggest a different

topology of the Na^+/H^+ antiporter in barley, as we do not expect protein phosphorylations inside the vacuole lumen due to the absence of ATP [28].

Sugar transport across the vacuolar membrane seems to be precisely controlled by protein phosphorylations, as numerous phosphopeptides of five sugar transporters were identified (Table 1). Interestingly, 12 phosphorylation sites were determined in the central cytosolic loop of HvSTP1/HvSTP2. The sugar transporters HvSTP1 and HvSTP2 are closely related (90% identity) and differ within the cytosolic loop in only one amino acid. Therefore, the identified phosphopeptides can not be attributed to one of both transporters. However, in the flow trough of the purified tonoplast fraction the unphosphorylated peptide LMDMSGR was detected, which is unique for HvSTP1 suggesting that the identified phosphopeptides derive from HvSTP1. The closest homologues in Arabidopsis of HvSTP1 and HvSTP2 is the putative tonoplast monosaccharide transporter 2 (TMT2) [35]. We aligned the central loop of HvSTP1 with the TMT family of Arabidopsis analysing the conservation of phosphorylation sites between monocotyledonous barley and dicotyledonous Arabidopsis (Figure 5). Five phosphorylation sites are conserved between HvSTP1 and all three members of the TMT family and even ten of twelve phosphosites between HvSTP1 and TMT2. Moreover, the amino acids surrounding the phosphorylation sites are highly conserved. Most likely phosphorylation sites identified here in barely can also be found in Arabidopsis. Analysis of the Arabidopsis tonoplast phosphoproteome is required to assess the conservation of phosphosites between dicotyledonous and monocotyledonous plants.

Several *in vivo* phosphorylation sites have been identified of plasma membrane localized aquaporins (PIPs) [36,13]. Prak et al. [13] could show that phosphorylation of AtPIP2;1 depends on salt stress and is important for the trafficking of the protein to the plasma membrane. Much less is known about the regulation of vacuolar aquaporins. Early studies have been shown that water channel activity of the storage vacuole-associated α-TIP could only be observed if a protein kinase was co-expressed in oocytes [37]. In a more recent study a *Phaseolus vulgaris* (Pv) TIP was expressed in *Pichia pastoris* and the overexpressed protein could be phosphorylated at S7 by a PKA [38]. Our results show that vacuolar aquaporins are phosphorylated at both the N-terminus as well as at the C-terminus. It will be interesting to analyse whether the two phosphorylation sites exhibit different functions.

Four phosphorylation sites have been identified in the two-pore (SV) channel, three of them are localized at the N-terminus. It has been shown in barley that 14-3-3 proteins rapidly reduce the SV currents [36]. Further analysis should reveal, which of the phosphosite is responsible for the 14-3-3 binding.

Finally, it may be interesting to note that in our phosphoproteomic study the closest homologue (gi_53791767) to AtALMT9 [40], a vacuolar malate channel, was identified in the tonoplast fraction. This channel has never been discovered in the different vacuolar proteomic approaches. This result indicates that the enrichment of phosphopeptides additionally allows the identification of previously unknown vacuolar membrane proteins.

The reported *in vivo* phosphorylation sites will provide a novel source to investigate the posttranslational regulation of vacuolar transporters and channels, in particular as most phosphorylation sites and their surrounding amino acids are highly conserved between barley and Arabidopsis. Further studies may characterize the function of the phosphorylations in controlling the targeting of the proteins to the tonoplast, the substrate specificity, the transport activity or protein-protein interaction of tonoplast proteins. In the future it will be interesting to analysis the dynamic of tonoplast phosphorylations in response to environmental changes and to search for protein kinases and phosphates involved in the vacuolar protein phosphorylation/dephosphorylation processes.

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Conflicts of Interest Statement

The authors declare to have no conflicts of interest.

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1169-1180.

Figure Legend

Figure 1

Western blot analysis comparing the quantity of thylakoid (LHC-a2; photosystem I light harvesting complex subunit 2 and LHC-b3; photosystem II light harvesting complex subunit 3, 27-28 kDa), tonoplast (γ-TIP; gamma-tonoplast intrinsic protein, 26 kDa) and plasma membrane proteins (PIP; plasma membrane intrinsic protein, 30 kDa) in the tonoplast fraction (To), tonoplast enriched microsomes (TEM) and total microsomes (TM).

Figure 2

Parallel approach of phosphopeptide enrichment, starting from purified tonoplast or tonoplast enriched microsomes of *Hordeum vulgare*.

Figure 3

Overlap of unambiguous phosphorylation sites of tonoplast proteins detected in the tonoplast fraction and tonoplast enriched microsomes (A) and organellar distribution of 428 phosphoproteins identified in the tonoplast enriched microsomes (B) and of 53 phosphoproteins identified in the tonoplast fraction (C). Percentages for tonoplast localization reflect 24 (B) and 23 phosphoproteins (C), respectively.

Figure 4

Relative frequency of amino acids surrounding unambiguous phosphorylation sites of tonoplast proteins from the +6 to -6 position.

Figure 5

Alignment of the central loop of the putative monosaccharide transporter HvSTP1 with the Arabidopsis TMT family. Phosphopeptides are marked with gray boxes. Phosphorylation sites are indicated in red and bold.

Supplementary Figure 1

Degree of phosphopeptide enrichment of the TEM fraction in the SCX-fractions 1-6 further enriched either by IMAC or TiO_2. The degree of enrichment reached by combining SCX and IMAC/TiO_2 is compared to the degree of enrichment using only IMAC or TiO_2. It is calculated the percentage of phosphopeptides among all identified peptides, holding at least a Mascot score of 25 and an ion expect value of ≤0.05 [23].

Supplementary Figure 2

Mascot spectra of phosphopeptides listed in Table 1.

Supplementary Table 1

Phosphopeptides enriched either by IMAC or TiO_2 affinity chromatography from tonoplast enriched microsomes (TEM) or purified tonoplast (Tono). Phosphorylation sites assigned by Mascot are indicated by a lower case (pS or pT). Information enclosed for phosphopeptides are: NCBI accession numbers (prot_acc), protein descriptions (prot_desc), peptide charges (pep_exp_z), the mass differences of the theoretical and experimental masses observed for the precursor ions (pep_delta), number of miss cleavage sites (pep_miss), Mascot peptides score (pep_score), Mascot p-value (pep_expect), peptide sequence (pep_seq), delta ion score (ΔI), variable modifications of the identified peptides (pep_var_mod) and subcellular

localization (sub_loca). The localization is indicated if experimental evidence for the subcellular localization of the protein or a close homologue is available.

Table 1. Identified phosphorylated peptide sequences of vacuolar membrane proteins in barley. Accession numbers are from NCBI or in case of EST sequences it is given the identifier of the consensus sequence from the IPK crop EST database. It is indicated from which starting material (tonoplast – Tono, tonoplast enriched microsomes – TEM) and by which techniques (IMAC or TiO_2) phosphopeptides were enriched. The phosphorylated amino acid is indicated by p (pS and pT).

Accession	Protein description	Phosphopeptide sequence	Δ ion score	TEM IMAC	TEM TiO_2	Tono IMAC	Tono TiO_2	phosphorylation site
Transporters								
gi_26986186/gi_26986188	hexose transporter, HvSTP [Hordeum vulgare subsp. vulgare]	**QATpSVEGK**	0.41	x	x			S382/S363
		QApTpSVEGK			x			T381/T362, S382/S363
		MPEVMGpSMR		x	x	x	x	S322/S303
		GGGQSALGpSALGLMSR	0.46	x	x	x	x	S283/S264
		GGGQSALGSALGLMpSR	0.40	x				S289/S270
		GGGQpSALGSALGLMSR	0.41	x	x			S279/S260
		GGGQpSALGpSALGLMSR	0.51	x	x			S279/S260, S283/S264
		GGGQpSALGSALGLMpSR	0.32	x	x			S279/S260, S289/S270
		GGGQSALGpSALGLMpSR	0.21	x	x			S283/S264, S289/S270
		IYLHEEGVpSGDR		x	x	x	x	S450/S431
		LYGAEEGVpSWIARPVR		x				S267/S248
		DLIEQQLAGPAMVHPpSEAVAK		x				S502/S483
		HGSMVpSQGK	0.40	x			x	S293/S274
		HGp*SMVSQGK	0.25	x				S296/S277
		HGpSMVpSQGK			x			S293/S274, S296/S277
		SLVDPLVTLFGpSVHEK	0.45			x		S311/S292
cl5700ct7116cn8330	putative hexose transporter (gi|49388943) [Oryza sativa (japonica cultivar-group)]	**GPSMLGSVLSLApSR**	0.31	x				S287#

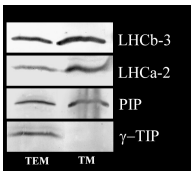
Accession	Protein description	Phosphopeptide sequence	Δ ion score	TEM IMAC	TEM TiO_2	Tono IMAC	Tono TiO_2	phosphorylation site
gi_51854311	putative sugar transporter [Oryza sativa (japonica cultivar-group)]	**TLEEIAFpSFR**	0.86	x				S499#
gi_51854288	putative sugar transporter [Oryza sativa (japonica cultivar-group)]	**TLEEIQWpSFR**	0.77	x				S499#
gi_7024413	sucrose transporter 2, HvSUT2 [Hordeum vulgare subsp. vulgare]	**RPNTGGGGGTSp*SSAAPAPR**	0.09	x		x		S16
gi_28201131	sodium/proton antiporter, HvNHX1 [Hordeum vulgare]	**FLLPASSNGDPSEPSp*SPK**	0.22	x	x	x	x	S461
		FLLPASSNGDPpSEPSp*SPK	0.34	x	x		x	S457, S461
		GFVPYSPGp*SPTDPNVIVA	0.11		x			T531
gi_ 124265192	vacuolar Na+/H+ antiporter, NHX2 [Hordeum vulgare]	**pSVHGPGLLGTVTEAENR**	0.67	x	x		x	S529
		GFVPFVPGpSPIER		x		x		S524
cl2215ct3225cn4100	Sodium/calcium exchanger protein (gi|56201517) [Oryza sativa (japonica cultivar-group)]	**VAASGAYp*SNKFVNDYHAR**	0.25	x				S392#
		VAASGAYp*SNK	0.28	x	x			S392#
		HVIpSGILQHAQK		x				S284#
gi_131054210	Ca2+/H+-exchanging protein [Hordeum vulgare subsp. vulgare]	**TAHGMSSSp*SLR**	0.23	x	x			S47
cl1841ct2785cn3589	putative sulphate transporter (gi_20162451) [Oryza sativa]	**LSPQASGHLTEpSPK**	0.52	x	x		x	S650#
		LpSPQASGHLp*TESPK	0.20	x	x		x	S640#, T648#
		LSPQAp*SGHLp*TESPK	0.13		x			S644#, T648#
		Lp*SPQAp*SGHLTEpSPK	0.22		x			S640#, S644#, S650#
		Lp*SPQASGHLTEp*SPK	0.12	x			x	S640#, S650#
gi_7108599	HAK2 [Hordeum vulgare subsp. vulgare]	**DSNEGTpSLTR**	0.34	x	x			S652

Accession	Protein description	Phosphopeptide sequence	Δ ion score	TEM IMAC	TEM TiO_2	Tono IMAC	Tono TiO_2	phosphorylation site
		FIQMEAEEAAp*SSGSYESSNEGR	0				x	S622
gi_75143740	HAK15_ORYSA Probable potassium transporter 15	**RMpSSFR**	0.33	x				S223#
		KAPpSMEWR		x				S24#
cl9992ct11668cn13180	amino acid transporter-like (gi|47497045) [Oryza sativa (japonica cultivar-group)]	**GEDGDGVGpSFESR**	0.60	x		x	x	S64#
Ion channels								
gi_39545849	two-pore calcium channel [Hordeum vulgare]	**GLASSGpSR**	0.38	x	x		x	S23
		GLASSGp*SRR	0.22	x			x	S23
		LSDGGGGQGpSRK	0.69	x	x			S35
		LSDGGGGQGpSR	0.39		x			S35
		RLSDGGGGQGpSR	0.73		x			S35
		RLpSDGGGGQGSR	0.72	x	x		x	S27
		GEEVDIQpSPTSGGIK	0.35	x	x	x	x	S703
gi_21321022	chloride channel [Oryza sativa (japonica cultivar-group)]	**TSGpSFVLR**	0.41	x	x	x		S694
		TSGpSFVLRR	0.44		x			S694
gi_53791767	aluminum-activated malate transporter-like [Oryza sativa (japonica cultivar-group)]	**pSFHPNLPLEDEETK**	0.83			x		S227#
Aquaporins								
gi_520936	gamma-TIP-like protein [Hordeum vulgare subsp. vulgare]	**IAVGpSHR**		x	x		x	S10
		IAVGpSHREVYEVGALK	0.88	x				S10
		THEQLPTp*TDY	0.17				x	S248

Accession	Protein description	Phosphopeptide sequence	Δ ion score	TEM IMAC	TEM TiO_2	Tono IMAC	Tono TiO_2	phosphorylation site
cl1115ct1919cn2576	delta tonoplast intrinsic protein TIP2 (gb_AAD10495.1) [Triticum aestivum]	**PGpSIAFGR**		x			x	S4#
Pumps								
gi_78707995/ gi_108707197	V-type ATPase 116kDa subunit family protein [Oryza sativa]	**DLNADKpSPFQR**		x	x	x	x	S55/S56
gi_11527563	vacuolar proton-ATPase-subunit A [Hordeum vulgare subsp. vulgare]	**MTTFEEp*SERESEYGYVR**	0.18	x			x	S13
		MTTFEEp*SER	0.25			x		S13
gi_167108	vacuolar ATPase B subunit [Hordeum vulgare]	**AVVQVFEGTp*SGIDNK**	0.16				x	S77
gi_6688428	vacuolar H+-ATPase subunit C [Hordeum vulgare subsp. vulgare]	**HpSFDTPLYR**	0.32				x	S36
cl8297ct9913cn11347	vacuolar ATP synthase subunit D (gi|38344879) [Oryza sativa]	**FTHFVDPAGASp*SPSLTGLAR**	0.15	x		x		S121#
cl2035ct3021cn3857	putative vacuolar ATP synthase subunit H (gi_28564802) [Oryza sativa (japonica cultivar-group)]	**YDHKPEpSQR**			x		x	S44
		LQNGMVPNGEASNp*SK	0.04			x		S151
gi_6012172	inorganic pyrophosphatase [Hordeum vulgare subsp. vulgare]	**VTPGAApSAAAGAK**	0.83	x	x	x	x	S42
		YIEAGNpSEHAR	0.81	x		x	x	S698
gi_11527561	vacuolar proton-inorganic pyrophosphatase, HVP1 [Hordeum vulgare subsp. vulgare]	**YIEAGApSEHAK**	1	x			x	S706
other proteins								
cl1952ct2915cn3735	senescence-associated protein-like (gi|37806408) [Oryza sativa (japonica cultivar-group)]	**AMNKPAEYDpSDDEIIGTAR**	0.53		x	x		S199#

*p indicates ambiguous phosphorylation sites

phosphorylation site according to rice or wheat protein sequence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LHCa-2
PIP
γ–TIP
TEM
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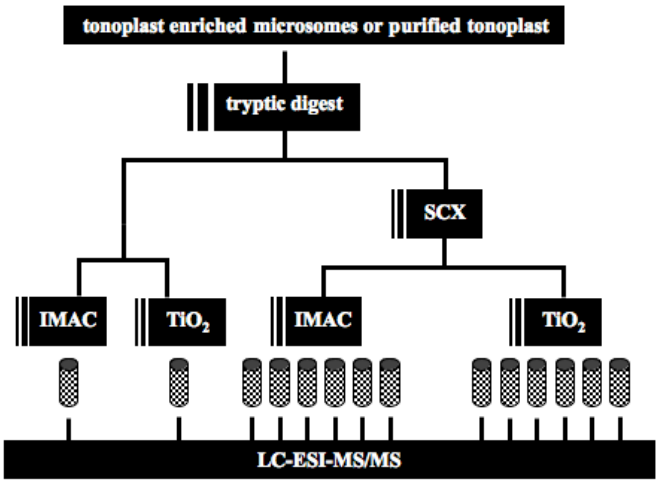
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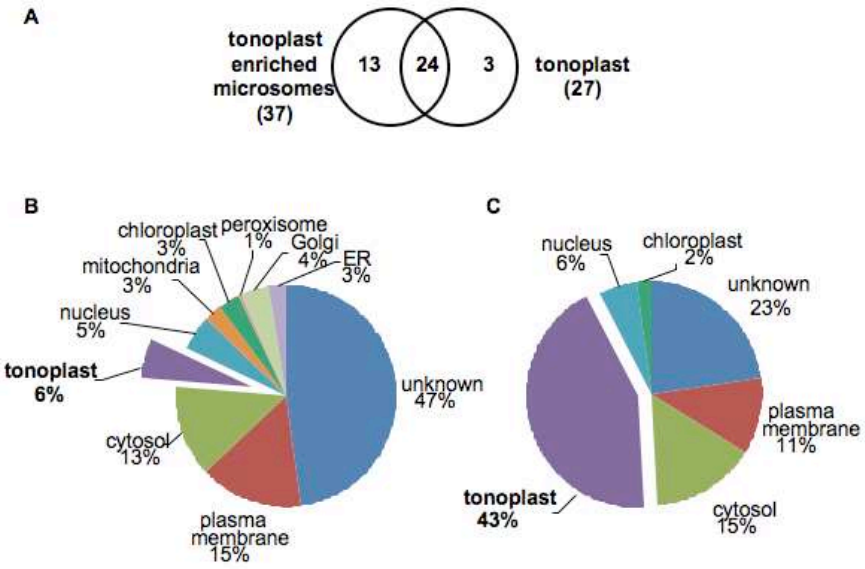
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nucleus 5%
tonoplast 6%
unknown 47%
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chloroplast 2%
unknown 23%
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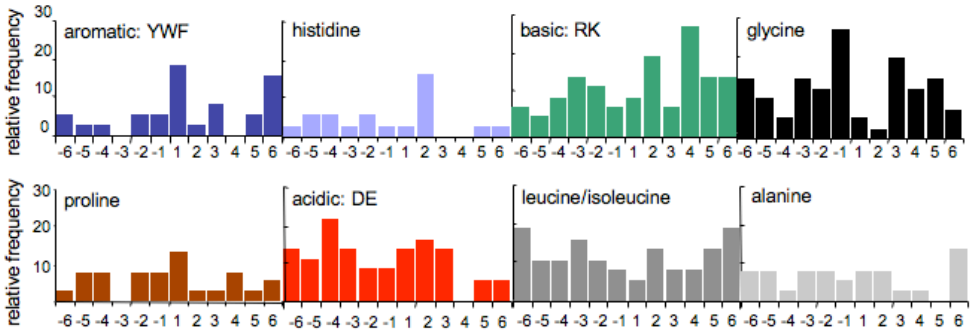
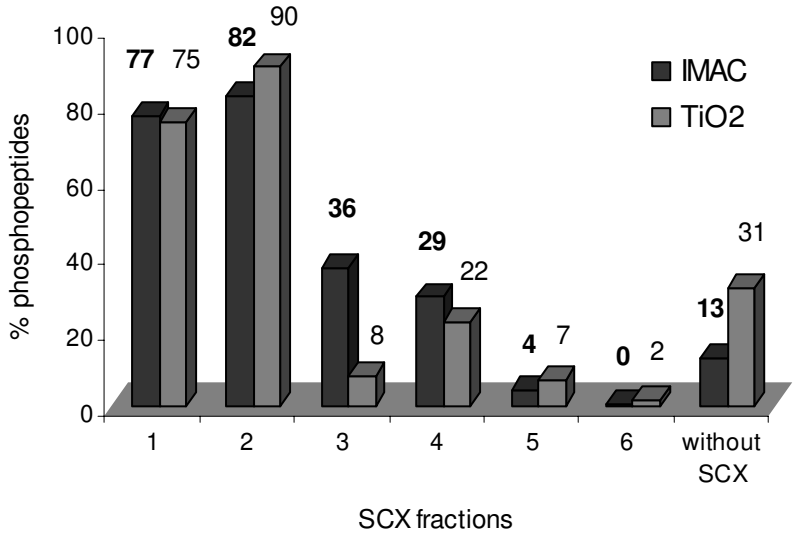
tonoplast 43%
cytosol 15%


relative frequency
30
20
10
0
aromatic: YWF
histidine
basic: RK
glycine
proline
acidic: DE
leucine/isoleucine
alanine
-6 -5 -4 -3 -2 -1 1 2 3 4 5 6


HvSTP1 PDDELADDG-LAPDQE-KLKLYGAEEGVSWIARPVRGGGQSALGSALGLMSRHGSMVSQGK-SLVDPLVTLFGSVHEKMP 317
AtTMT2 PADEVTDDHDIAVDKD-QIKLYGAEEGLSWVARPVK-GG-----STMSVLSRHGSTMSRRQGSLIDPLVTLFGSVHEKMP 312
AtTMT3 PDNEENEGGNELPRKD-QIKLYGPEDGQSWMAKPVKGQS------SLALASRQGSMLPRGG-SLMDPLVTLFGSIHENLP 311
AtTMT1 LEDHEGDDTLETVDEDGQMRLYGTHENQSYLARPVPEQN-----SSLGLRSRHGSLANQSM-ILKDPLVNLFGSLHEKMP 311
eq
HvSTP1 --EVMGSMRSTLFPNFGSMFSVAEQQQA--KADWD---AESHRDD-EDYASDHGA---DDIEDNLNSPLISRQATSVEGK 386
AtTMT2 --DT-GSMRSALFPHFGSMFSVGGNQPR--HEDWD---EENLVGEGEDYPSDHG----DDSEDDLHSPLISRQTTSMEKD 380
AtTMT3 SENMNASSRSMLFPNMGSILGMMGRQES----QWD------PERNNEDSS---------DQDENLNSPLLSPQTTEPD-D 371
AtTMT1 --EAGGNTRSGIFPHFGSMFSTTADAPHGKPAHWEKDIESHYNKDNDDYATDDGAGDDDDSDNDLRSPLMSRQTTSMDKD 389
eq
HvSTP1 EIAAPHG--SIMGGVESSSMQG--GDAVSSMGIGGGWQLAWKWTEREGADGQKE-GGFQRIYLHEEGVSG-------DRR 454
AtTMT2 MPHTAHG--TLSTFRHGSQVQGAQGEGAGSMGIGGGWQVAWKWTEREDESGQKE-GGFKRIYLHQEGFPG-------SRR 450
AtTMT3 YHQRTVG---TMHRRQSSLFMANVGETATATSIGGGWQLAWKYNDKVGADGKRVNGGLQRMYIHEETANNNTNNIPFSRR 448
AtTMT1 MIPHPTSGSTLSMRRHSTLMQG---NGESSMGIGGGWHMGYRYENDE----------YKRYYLKEDGAE--------SRR 448
eq
HvSTP1 GSILSMP--GGDIPPGGEYIQAAALVSQPALYSKDLIEQQLAGPAMVHPSEAVAKGTKWAELFEPGVKHALFVGIGLQIL 532
AtTMT2 GSIVSLP--GGDGTGEADFVQASALVSQPALYSKDLLKEHTIGPAMVHPSETT-KGSIWHDLHDPGVKRALVVGVGLQIL 527
AtTMT3 GSLLSFHPEGDGHDQVNGYVQAAALVSQASMMPG------GKGETAMLPKEVK-DGPGWRELKEPGVKRALMVGVGLQIL 521
AtTMT1 GSIISIP--GGP-DGGGSYIHASALVSRSVLGPK-----SVHGSAMVPPEKIAASGPLWSALLEPGVKRALVVGVGIQIL 520
eq

Supplementary Figure 1



MS/MS spectra for phosphopeptides in Table 1

pS: phosphoserine

pT: phosphothreonine

p*S/p*T indicates ambiguous phosphorylation site

1 denotes $[M+2H^+-98]^{2+}$ (in some cases the neutral loss peak and fragment ions obtain the same mass)

2 denotes $[M+2H^+-18]^{2+}$

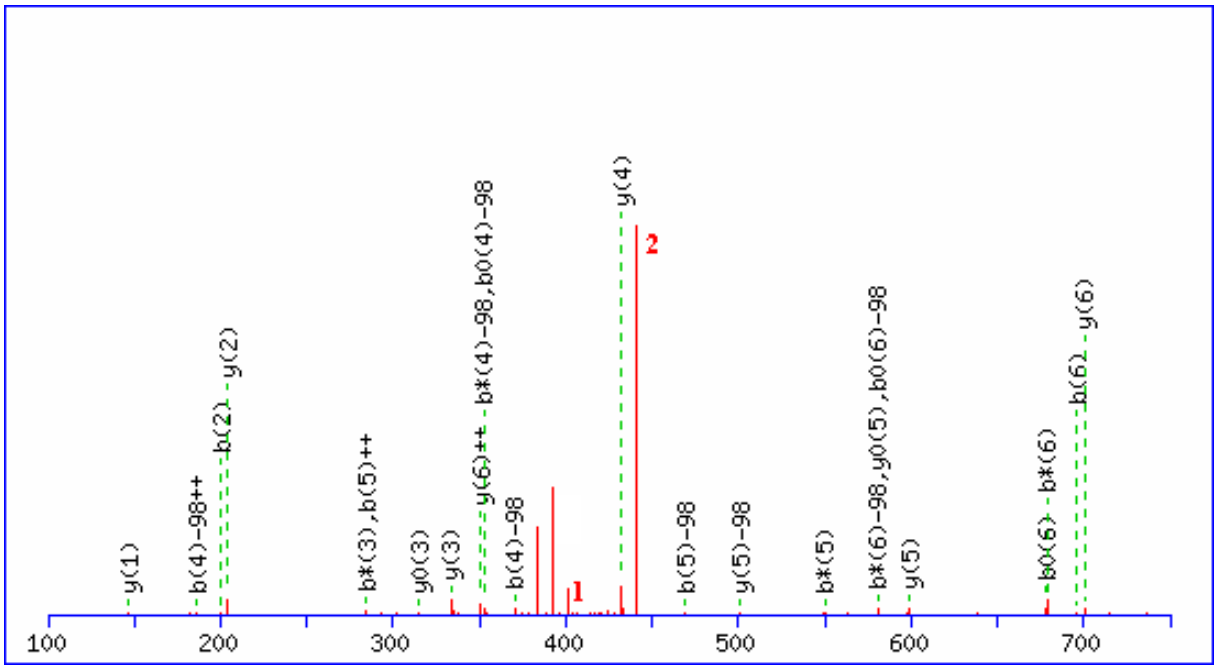
3 denotes $[M+2H^+-196]^{2+}$

4 denotes $[M+2H^+-196-18]^{2+}$

5 denotes $[M+2H^+-98-18]^{2+}$

6 denotes $[M+3H^+-98]^{3+}$

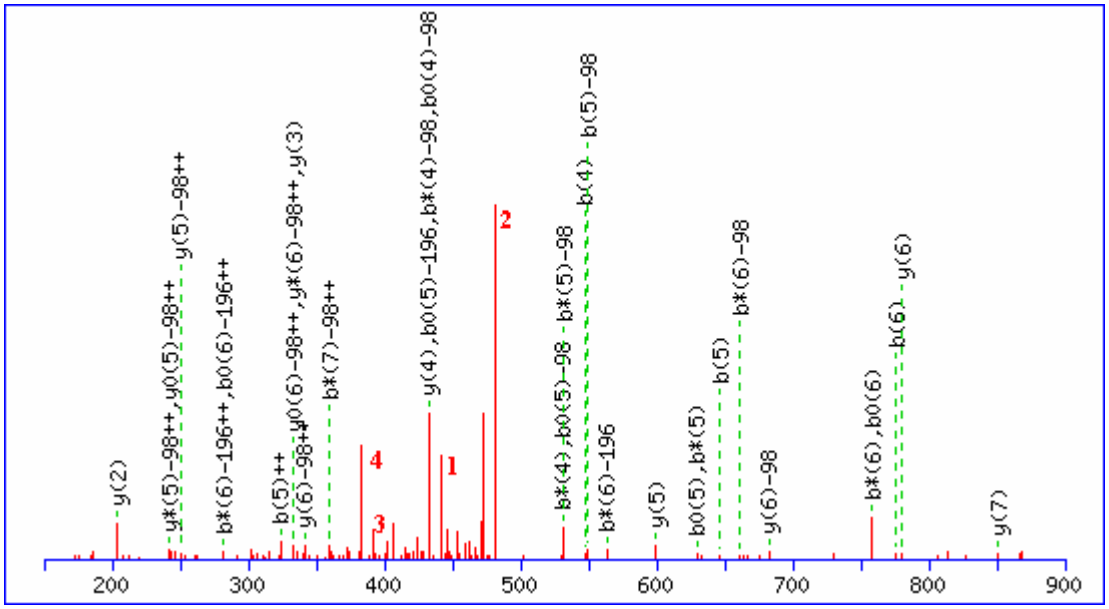
MS/MS Fragmentation of **QATpSVEGK**



Monoisotopic mass of neutral peptide Mr(calc): 898.3797
Variable modifications:
S4 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 56 **Expect:** 9.9e-05
Matches (Bold Red): 24/118 fragment ions using 25 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	129.0659	65.0366	112.0393	56.5233			Q							8
2	**200.1030**	100.5551	183.0764	92.0418			A	771.3284	386.1678	754.3019	377.6546	753.3179	377.1626	7
3	301.1506	151.0790	**284.1241**	142.5657	283.1401	142.0737	T	**700.2913**	**350.6493**	683.2648	342.1360	682.2807	341.6440	6
4	468.1490	234.5781	451.1224	226.0649	450.1384	225.5729	S	**599.2436**	300.1255	582.2171	291.6122	**581.2331**	291.1202	5
5	567.2174	**284.1123**	**550.1909**	275.5991	549.2068	275.1071	V	**432.2453**	216.6263	415.2187	208.1130	414.2347	207.6210	4
6	**696.2600**	348.6336	**679.2335**	340.1204	**678.2494**	339.6284	E	**333.1769**	167.0921	316.1503	158.5788	**315.1663**	158.0868	3
7	753.2815	377.1444	736.2549	368.6311	735.2709	368.1391	G	**204.1343**	102.5708	187.1077	94.0575			2
8							K	**147.1128**	74.0600	130.0863	65.5468			1

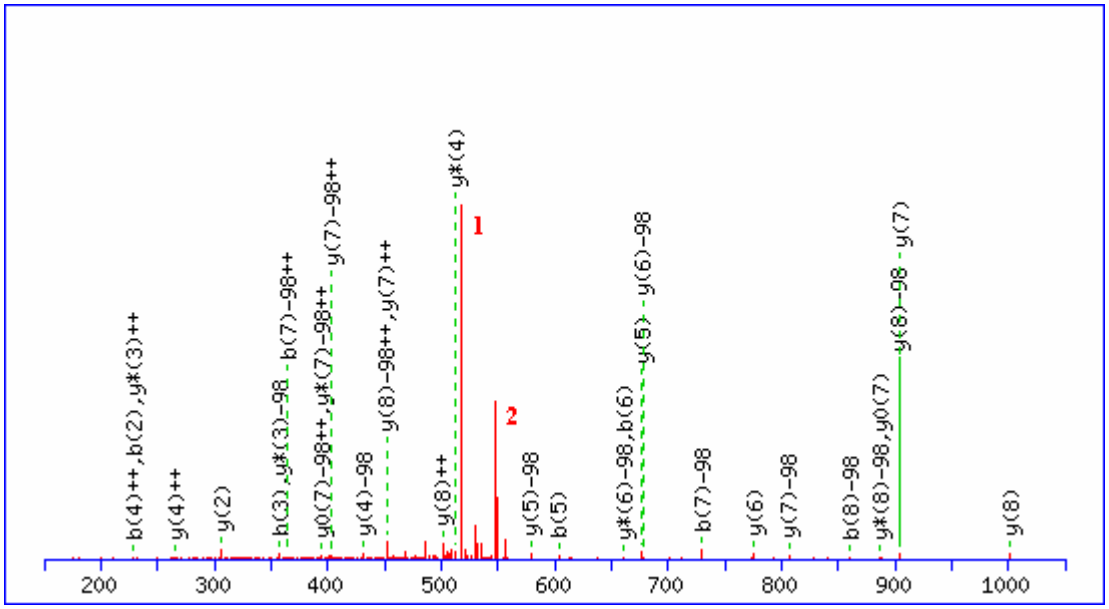
MS/MS Fragmentation of **QApTpSVEGK**



Monoisotopic mass of neutral peptide Mr(calc): 978.3460
Variable modifications:
T3 : Phospho (T), with neutral losses 0.0000(shown in table), 97.9769
S4 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 30 **Expect:** 0.016
Matches (Bold Red): 44/196 fragment ions using 48 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	129.0659	65.0366	112.0393	56.5233			Q							8
2	200.1030	100.5551	183.0764	92.0418			A	**851.2947**	426.1510	834.2682	417.6377	833.2842	417.1457	7
3	381.1170	191.0621	364.0904	182.5488	363.1064	182.0568	T	**780.2576**	390.6325	763.2311	382.1192	762.2471	381.6272	6
4	**548.1153**	274.5613	**531.0888**	266.0480	530.1048	265.5560	S	**599.2436**	300.1255	582.2171	291.6122	581.2331	291.1202	5
5	**647.1837**	**324.0955**	**630.1572**	315.5822	**629.1732**	315.0902	V	**432.2453**	216.6263	415.2187	208.1130	414.2347	207.6210	4
6	**776.2263**	388.6168	**759.1998**	380.1035	**758.2158**	379.6115	E	**333.1769**	167.0921	316.1503	158.5788	315.1663	158.0868	3
7	833.2478	417.1275	816.2212	408.6143	815.2372	408.1223	G	**204.1343**	102.5708	187.1077	94.0575			2
8							K	147.1128	74.0600	130.0863	65.5468			1

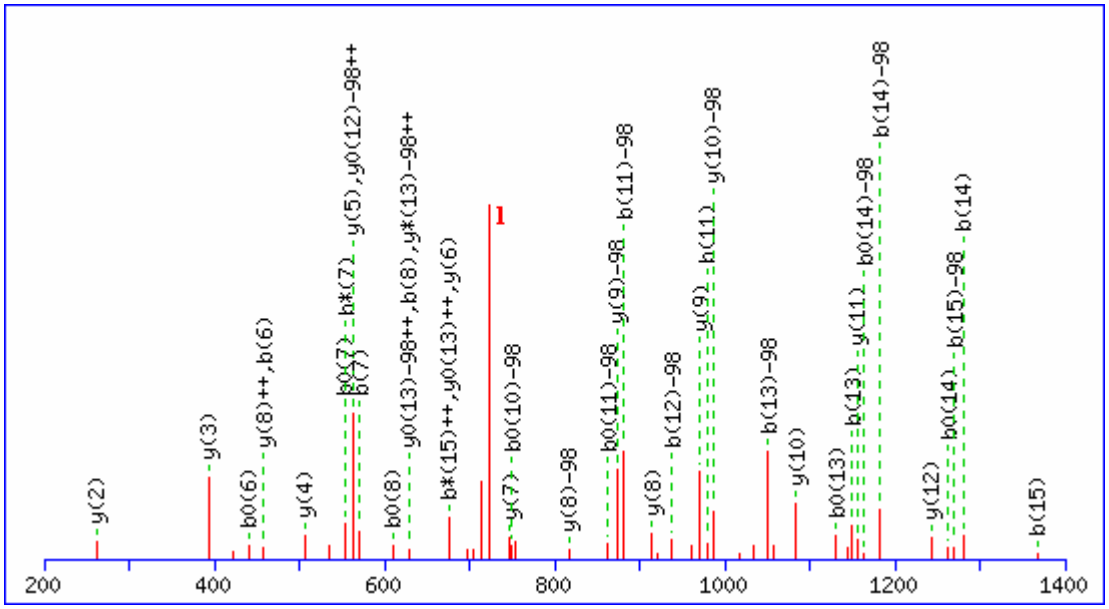
MS/MS Fragmentation of **MPEVMGpSMR**



Monoisotopic mass of neutral peptide Mr(calc): 1132.4116
Variable modifications:
M5 : Oxidation (M)
S7 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 56 **Expect:** 3.2e-05
Matches (Bold Red): 31/116 fragment ions using 37 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	132.0478	66.5275			M							9
2	**229.1005**	115.0539			P	**904.4015**	**452.7044**	**887.3750**	444.1911	886.3909	443.6991	8
3	**358.1431**	179.5752	340.1325	170.5699	E	**807.3487**	**404.1780**	790.3222	**395.6647**	789.3382	**395.1727**	7
4	457.2115	**229.1094**	439.2010	220.1041	V	**678.3062**	339.6567	**661.2796**	331.1434	660.2956	330.6514	6
5	**604.2469**	302.6271	586.2364	293.6218	M	**579.2377**	290.1225	562.2112	281.6092	561.2272	281.1172	5
6	**661.2684**	331.1378	643.2578	322.1325	G	**432.2023**	216.6048	415.1758	208.0915	414.1918	207.5995	4
7	**730.2898**	**365.6486**	712.2793	356.6433	S	375.1809	188.0941	**358.1543**	179.5808	357.1703	179.0888	3
8	**861.3303**	431.1688	843.3197	422.1635	M	**306.1594**	153.5834	289.1329	145.0701			2
9					R	175.1190	88.0631	158.0924	79.5498			1

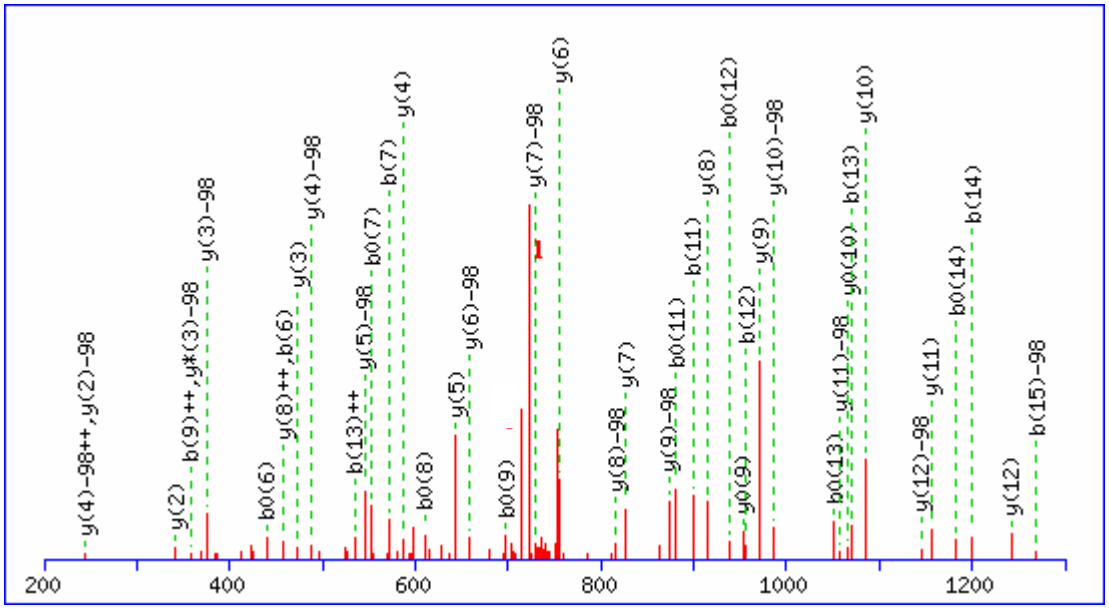
MS/MS Fragmentation of **GGGQSALGpSALGLMSR**



Monoisotopic mass of neutral peptide Mr(calc): 1540.7069
Variable modifications:
S9 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 109 **Expect:** 7.7e-10
Matches (Bold Red): 41/254 fragment ions using 40 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180					G							16
2	115.0502	58.0287					G	1484.6927	742.8500	1467.6662	734.3367	1466.6821	733.8447	15
3	172.0717	86.5395					G	1427.6712	714.3393	1410.6447	705.8260	1409.6607	705.3340	14
4	300.1302	150.5688	283.1037	142.0555			Q	1370.6498	685.8285	1353.6232	677.3152	1352.6392	**676.8232**	13
5	387.1623	194.0848	370.1357	185.5715	369.1517	185.0795	S	**1242.5912**	621.7992	1225.5646	613.2860	1224.5806	612.7940	12
6	**458.1994**	229.6033	441.1728	221.0901	**440.1888**	220.5980	A	**1155.5592**	578.2832	1138.5326	569.7699	1137.5486	569.2779	11
7	**571.2835**	286.1454	**554.2569**	277.6321	**553.2729**	277.1401	L	**1084.5221**	542.7647	1067.4955	534.2514	1066.5115	533.7594	10
8	**628.3049**	314.6561	611.2784	306.1428	**610.2944**	305.6508	G	**971.4380**	486.2226	954.4114	477.7094	953.4274	477.2173	9
9	795.3033	398.1553	778.2767	389.6420	777.2927	389.1500	S	**914.4165**	**457.7119**	897.3900	449.1986	896.4060	448.7066	8
10	866.3404	433.6738	849.3138	425.1606	848.3298	424.6685	A	**747.4182**	374.2127	730.3916	365.6994	729.4076	365.2074	7
11	**979.4244**	490.2159	962.3979	481.7026	961.4139	481.2106	L	**676.3811**	338.6942	659.3545	330.1809	658.3705	329.6889	6
12	1036.4459	518.7266	1019.4194	510.2133	1018.4353	509.7213	G	**563.2970**	282.1521	546.2704	273.6389	545.2864	273.1469	5
13	**1149.5300**	575.2686	1132.5034	566.7554	**1131.5194**	566.2633	L	**506.2755**	253.6414	489.2490	245.1281	488.2650	244.6361	4
14	**1280.5705**	640.7889	1263.5439	632.2756	**1262.5599**	631.7836	M	**393.1915**	197.0994	376.1649	188.5861	375.1809	188.0941	3
15	**1367.6025**	684.3049	1350.5759	**675.7916**	1349.5919	675.2996	S	**262.1510**	131.5791	245.1244	123.0659	244.1404	122.5738	2
16							R	175.1190	88.0631	158.0924	79.5498			1

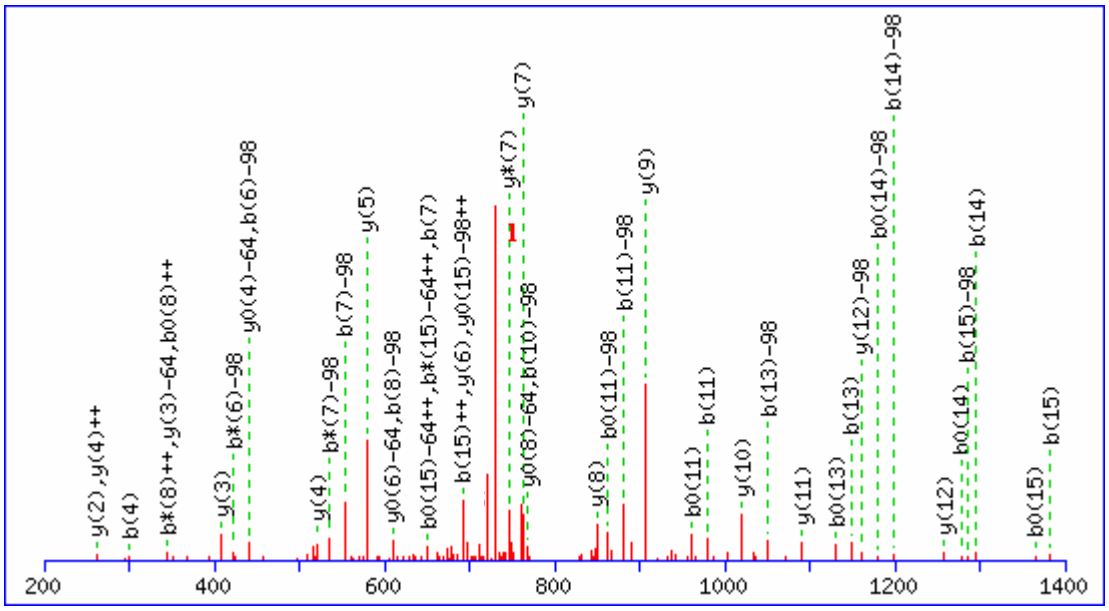
MS/MS Fragmentation of **GGGQSALGSALGLMpSR**



Monoisotopic mass of neutral peptide Mr(calc): 1540.7069
Variable modifications:
S15 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 82 **Expect:** 3.9e-07
Matches (Bold Red): 44/254 fragment ions using 59 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180					G							16
2	115.0502	58.0287					G	1484.6927	742.8500	1467.6662	734.3367	1466.6821	733.8447	15
3	172.0717	86.5395					G	1427.6712	714.3393	1410.6447	705.8260	1409.6607	705.3340	14
4	300.1302	150.5688	283.1037	142.0555			Q	1370.6498	685.8285	1353.6232	677.3152	1352.6392	676.8232	13
5	387.1623	194.0848	370.1357	185.5715	369.1517	185.0795	S	**1242.5912**	621.7992	1225.5646	613.2860	1224.5806	612.7940	12
6	**458.1994**	229.6033	441.1728	221.0901	**440.1888**	220.5980	A	**1155.5592**	578.2832	1138.5326	569.7699	1137.5486	569.2779	11
7	**571.2835**	286.1454	554.2569	277.6321	**553.2729**	277.1401	L	**1084.5221**	542.7647	1067.4955	534.2514	**1066.5115**	533.7594	10
8	628.3049	314.6561	611.2784	306.1428	**610.2944**	305.6508	G	**971.4380**	486.2226	954.4114	477.7094	**953.4274**	477.2173	9
9	715.3369	**358.1721**	698.3104	349.6588	**697.3264**	349.1668	S	**914.4165**	**457.7119**	897.3900	449.1986	896.4060	448.7066	8
10	786.3741	393.6907	769.3475	385.1774	768.3635	384.6854	A	**827.3845**	414.1959	810.3579	405.6826	809.3739	405.1906	7
11	**899.4581**	450.2327	882.4316	441.7194	**881.4476**	441.2274	L	**756.3474**	378.6773	739.3208	370.1641	738.3368	369.6720	6
12	**956.4796**	478.7434	939.4530	470.2302	**938.4690**	469.7381	G	**643.2633**	322.1353	626.2368	313.6220	625.2528	313.1300	5
13	**1069.5637**	**535.2855**	1052.5371	526.7722	**1051.5531**	526.2802	L	**586.2419**	293.6246	569.2153	285.1113	568.2313	284.6193	4
14	**1200.6041**	600.8057	1183.5776	592.2924	**1182.5936**	591.8004	M	**473.1578**	237.0825	456.1312	228.5693	455.1472	228.0772	3
15	1367.6025	684.3049	1350.5759	675.7916	1349.5919	675.2996	S	**342.1173**	171.5623	325.0908	163.0490	324.1067	162.5570	2
16							R	175.1190	88.0631	158.0924	79.5498			1

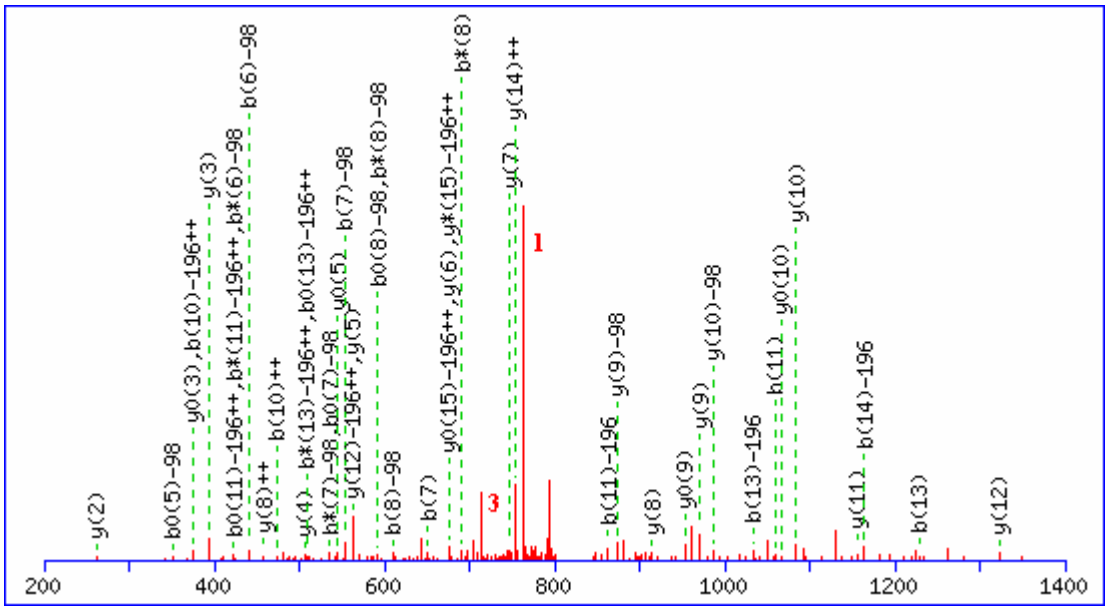
MS/MS Fragmentation of **GGGQpSALGSALGLMSR**



Monoisotopic mass of neutral peptide Mr(calc): 1556.7018
Variable modifications:
S5 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
M14 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
Ions Score: 104 **Expect:** 2.3e-09
Matches (Bold Red): 46/380 fragment ions using 39 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180					G							16
2	115.0502	58.0287					G	1500.6876	750.8474	1483.6611	742.3342	1482.6771	741.8422	15
3	172.0717	86.5395					G	1443.6662	722.3367	1426.6396	713.8234	1425.6556	713.3314	14
4	**300.1302**	150.5688	283.1037	142.0555			Q	1386.6447	693.8260	1369.6181	685.3127	1368.6341	684.8207	13
5	467.1286	234.0679	450.1020	225.5547	449.1180	225.0627	S	**1258.5861**	629.7967	1241.5596	621.2834	1240.5755	620.7914	12
6	538.1657	269.5865	521.1392	261.0732	520.1551	260.5812	A	**1091.5878**	546.2975	1074.5612	537.7842	1073.5772	537.2922	11
7	**651.2498**	326.1285	634.2232	317.6153	633.2392	317.1232	L	**1020.5506**	510.7790	1003.5241	502.2657	1002.5401	501.7737	10
8	708.2712	354.6393	691.2447	**346.1260**	690.2607	**345.6340**	G	**907.4666**	454.2369	890.4400	445.7237	889.4560	445.2316	9
9	795.3033	398.1553	778.2767	389.6420	777.2927	389.1500	S	**850.4451**	425.7262	833.4186	417.2129	832.4346	416.7209	8
10	866.3404	433.6738	849.3138	425.1606	848.3298	424.6685	A	**763.4131**	382.2102	**746.3865**	373.6969	745.4025	373.2049	7
11	**979.4244**	490.2159	962.3979	481.7026	**961.4139**	481.2106	L	**692.3760**	346.6916	675.3494	338.1784	674.3654	337.6863	6
12	1036.4459	518.7266	1019.4194	510.2133	1018.4353	509.7213	G	**579.2919**	290.1496	562.2654	281.6363	561.2813	281.1443	5
13	**1149.5300**	575.2686	1132.5034	566.7554	**1131.5194**	566.2633	L	**522.2704**	**261.6389**	505.2439	253.1256	504.2599	252.6336	4
14	**1296.5654**	648.7863	1279.5388	640.2731	**1278.5548**	639.7810	M	**409.1864**	205.0968	392.1598	196.5836	391.1758	196.0915	3
15	**1383.5974**	**692.3023**	1366.5709	683.7891	**1365.5868**	683.2971	S	**262.1510**	131.5791	245.1244	123.0659	244.1404	122.5738	2
16							R	175.1190	88.0631	158.0924	79.5498			1

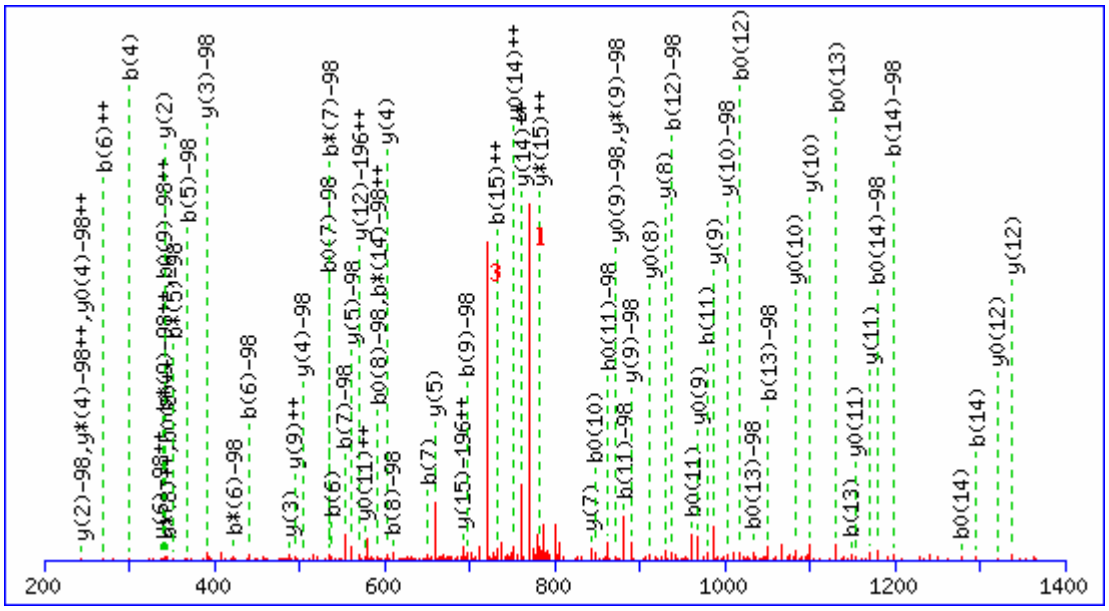
MS/MS Fragmentation of **GGGQpSALGpSALGLMSR**



Monoisotopic mass of neutral peptide Mr(calc): 1620.6732
Variable modifications:
S5 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
S9 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 60 **Expect:** 3.9e-05
Matches (Bold Red): 44/278 fragment ions using 62 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180					G							16
2	115.0502	58.0287					G	1564.6590	782.8331	1547.6325	774.3199	1546.6485	773.8279	15
3	172.0717	86.5395					G	1507.6376	**754.3224**	1490.6110	745.8091	1489.6270	745.3171	14
4	300.1302	150.5688	283.1037	142.0555			Q	1450.6161	725.8117	1433.5895	717.2984	1432.6055	716.8064	13
5	467.1286	234.0679	450.1020	225.5547	449.1180	225.0627	S	**1322.5575**	661.7824	1305.5310	653.2691	1304.5470	652.7771	12
6	538.1657	269.5865	521.1392	261.0732	520.1551	260.5812	A	**1155.5592**	578.2832	1138.5326	569.7699	1137.5486	569.2779	11
7	**651.2498**	326.1285	634.2232	317.6153	633.2392	317.1232	L	**1084.5221**	542.7647	1067.4955	534.2514	**1066.5115**	533.7594	10
8	708.2712	354.6393	**691.2447**	346.1260	690.2607	345.6340	G	**971.4380**	486.2226	954.4114	477.7094	**953.4274**	477.2173	9
9	875.2696	438.1384	858.2430	429.6252	857.2590	429.1332	S	**914.4165**	**457.7119**	897.3900	449.1986	896.4060	448.7066	8
10	946.3067	**473.6570**	929.2802	465.1437	928.2961	464.6517	A	**747.4182**	374.2127	730.3916	365.6994	729.4076	365.2074	7
11	**1059.3908**	530.1990	1042.3642	521.6857	1041.3802	521.1937	L	**676.3811**	338.6942	659.3545	330.1809	658.3705	329.6889	6
12	1116.4122	558.7098	1099.3857	550.1965	1098.4017	549.7045	G	**563.2970**	282.1521	546.2704	273.6389	**545.2864**	273.1469	5
13	**1229.4963**	615.2518	1212.4697	606.7385	1211.4857	606.2465	L	**506.2755**	253.6414	489.2490	245.1281	488.2650	244.6361	4
14	1360.5368	680.7720	1343.5102	672.2588	1342.5262	671.7667	M	**393.1915**	197.0994	376.1649	188.5861	**375.1809**	188.0941	3
15	1447.5688	724.2880	1430.5423	715.7748	1429.5582	715.2828	S	**262.1510**	131.5791	245.1244	123.0659	244.1404	122.5738	2
16							R	175.1190	88.0631	158.0924	79.5498			1

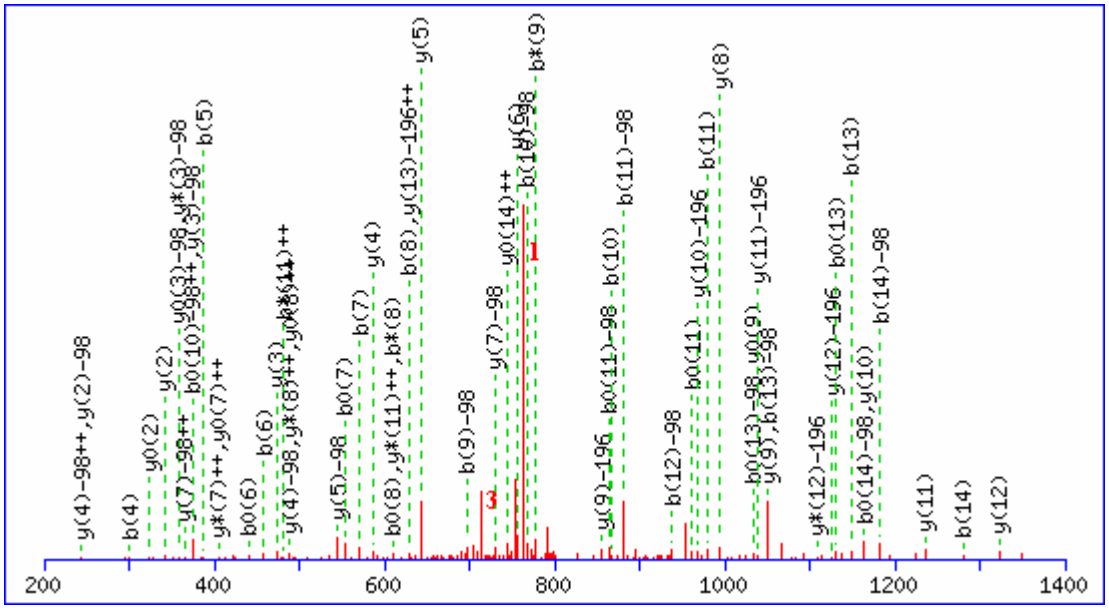
MS/MS Fragmentation of **GGGQpSALGSALGLMpSR**



Monoisotopic mass of neutral peptide Mr(calc): 1636.6680
Variable modifications:
S5 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
M14 : Oxidation (M)
S15 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 37 **Expect:** 0.0086
Matches (Bold Red): 68/314 fragment ions using 102 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180					G							16
2	115.0502	58.0287					G	1384.7001	**692.8537**	1367.6736	684.3404	1366.6896	683.8484	15
3	172.0717	86.5395					G	1327.6787	664.3430	1310.6521	655.8297	1309.6681	655.3377	14
4	**300.1302**	150.5688	283.1037	142.0555			Q	1270.6572	635.8322	1253.6306	627.3190	1252.6466	626.8270	13
5	**369.1517**	185.0795	**352.1251**	176.5662	351.1411	176.0742	S	1142.5986	**571.8029**	1125.5721	563.2897	1124.5881	562.7977	12
6	**440.1888**	220.5980	**423.1623**	212.0848	422.1782	211.5928	A	1073.5772	537.2922	1056.5506	528.7789	1055.5666	528.2869	11
7	**553.2729**	277.1401	**536.2463**	268.6268	**535.2623**	268.1348	L	**1002.5400**	501.7737	985.5135	493.2604	984.5295	492.7684	10
8	**610.2943**	305.6508	593.2678	297.1375	**592.2838**	296.6455	G	**889.4560**	445.2316	**872.4294**	436.7184	**871.4454**	436.2263	9
9	**697.3264**	349.1668	680.2998	**340.6535**	679.3158	**340.1615**	S	832.4345	416.7209	815.4080	408.2076	814.4240	407.7156	8
10	768.3635	384.6854	751.3369	376.1721	750.3529	375.6801	A	745.4025	373.2049	728.3759	364.6916	727.3919	364.1996	7
11	**881.4475**	441.2274	864.4210	432.7141	**863.4370**	432.2221	L	674.3654	**337.6863**	657.3388	329.1731	656.3548	328.6810	6
12	**938.4690**	469.7381	921.4424	461.2249	920.4584	460.7328	G	**561.2813**	281.1443	544.2548	272.6310	543.2708	272.1390	5
13	**1051.5530**	526.2802	1034.5265	517.7669	**1033.5425**	517.2749	L	**504.2599**	252.6336	487.2333	**244.1203**	486.2493	**243.6283**	4
14	**1198.5884**	599.7979	1181.5619	**591.2846**	**1180.5779**	590.7926	M	**391.1758**	196.0915	374.1493	187.5783	373.1652	187.0863	3
15	1267.6099	634.3086	1250.5834	625.7953	1249.5993	625.3033	S	**244.1404**	122.5738	227.1139	114.0606	226.1298	113.5686	2
16							R	175.1190	88.0631	158.0924	79.5498			1

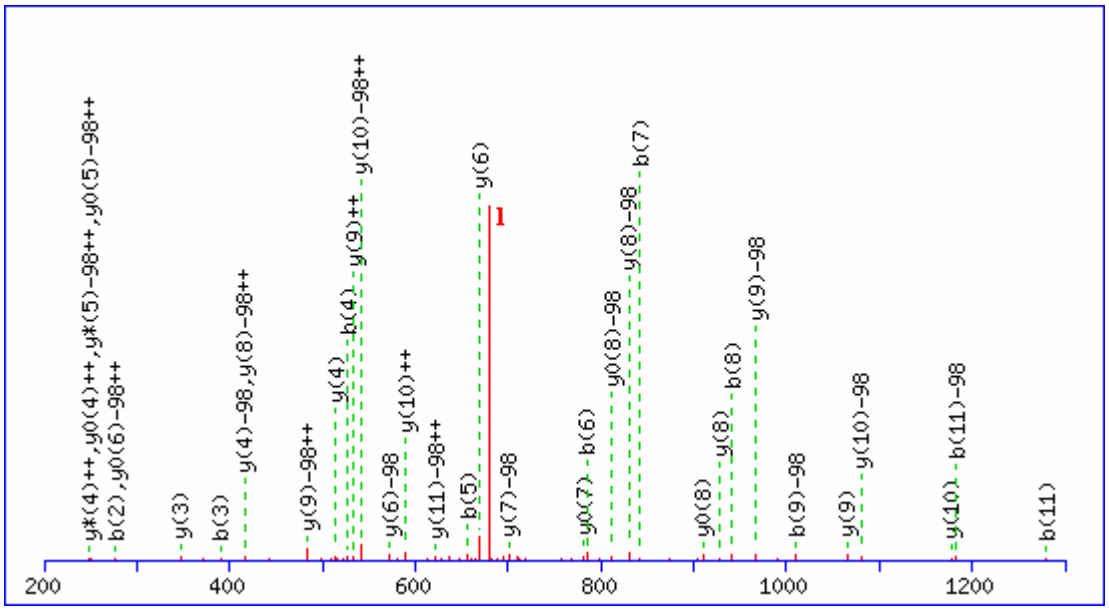
MS/MS Fragmentation of **GGGQSALGpSALGLMpSR**



Monoisotopic mass of neutral peptide Mr(calc): 1620.6731
Variable modifications:
S9 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
S15 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 58 **Expect:** 6.5e-05
Matches (Bold Red): 60/290 fragment ions using 79 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180					G							16
2	115.0502	58.0287					G	1368.7052	684.8562	1351.6786	676.3430	1350.6946	675.8510	15
3	172.0717	86.5395					G	1311.6837	656.3455	1294.6572	647.8322	1293.6732	647.3402	14
4	**300.1302**	150.5688	283.1037	142.0555			Q	1254.6623	**627.8348**	1237.6357	619.3215	1236.6517	618.8295	13
5	**387.1623**	194.0848	370.1357	185.5715	369.1517	185.0795	S	**1126.6037**	563.8055	**1109.5771**	555.2922	1108.5931	554.8002	12
6	**458.1994**	229.6033	441.1728	221.0901	**440.1888**	220.5980	A	**1039.5717**	520.2895	1022.5451	511.7762	1021.5611	511.2842	11
7	**571.2834**	286.1454	554.2569	277.6321	**553.2729**	277.1401	L	**968.5346**	484.7709	951.5080	476.2576	950.5240	475.7656	10
8	**628.3049**	314.6561	**611.2783**	306.1428	**610.2943**	305.6508	G	**855.4505**	428.2289	838.4239	419.7156	837.4399	419.2236	9
9	**697.3264**	349.1668	680.2998	340.6535	679.3158	340.1615	S	798.4290	399.7182	781.4025	391.2049	780.4185	390.7129	8
10	**768.3635**	384.6854	751.3369	376.1721	750.3529	**375.6801**	A	**729.4076**	**365.2074**	712.3810	356.6942	711.3970	356.2021	7
11	**881.4475**	441.2274	864.4210	432.7141	**863.4370**	432.2221	L	658.3705	329.6889	641.3439	321.1756	640.3599	320.6836	6
12	**938.4690**	469.7381	921.4424	461.2249	920.4584	460.7328	G	**545.2864**	273.1468	528.2599	264.6336	527.2758	264.1416	5
13	**1051.5530**	526.2802	1034.5265	517.7669	**1033.5425**	517.2749	L	**488.2649**	**244.6361**	471.2384	236.1228	470.2544	235.6308	4
14	**1182.5935**	591.8004	1165.5670	583.2871	**1164.5830**	582.7951	M	**375.1809**	188.0941	**358.1543**	179.5808	**357.1703**	179.0888	3
15	1251.6150	626.3111	1234.5884	617.7979	1233.6044	617.3058	S	**244.1404**	122.5738	227.1139	114.0606	226.1298	113.5686	2
16							R	175.1190	88.0631	158.0924	79.5498			1

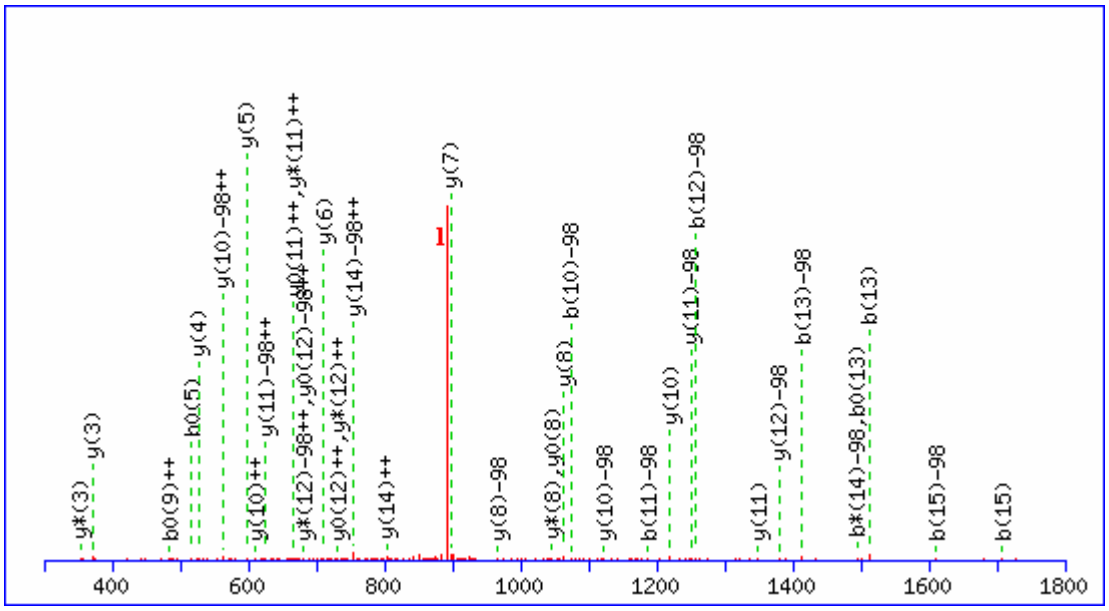
MS/MS Fragmentation of **IYLHEEGVpSGDR**



Monoisotopic mass of neutral peptide Mr(calc): 1453.6238
Variable modifications:
S9 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 69 **Expect:** 5.8e-06
Matches (Bold Red): 36/160 fragment ions using 41 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493			I							12
2	**277.1547**	139.0810			Y	1243.5702	**622.2887**	1226.5436	613.7754	1225.5596	613.2834	11
3	**390.2387**	195.6230			L	**1080.5068**	**540.7571**	1063.4803	532.2438	1062.4963	531.7518	10
4	**527.2976**	264.1525			H	**967.4228**	**484.2150**	950.3962	475.7017	949.4122	475.2097	9
5	**656.3402**	328.6738	638.3297	319.6685	E	**830.3639**	**415.6856**	813.3373	407.1723	**812.3533**	406.6803	8
6	**785.3828**	393.1950	767.3723	384.1898	E	**701.3213**	351.1643	684.2947	342.6510	683.3107	342.1590	7
7	**842.4043**	421.7058	824.3937	412.7005	G	**572.2787**	286.6430	555.2521	278.1297	554.2681	**277.6377**	6
8	**941.4727**	471.2400	923.4621	462.2347	V	515.2572	258.1322	498.2307	**249.6190**	497.2467	**249.1270**	5
9	**1010.4941**	505.7507	992.4836	496.7454	S	**416.1888**	208.5980	399.1623	200.0848	398.1782	199.5928	4
10	1067.5156	534.2614	1049.5050	525.2562	G	**347.1674**	174.0873	330.1408	165.5740	329.1568	165.0820	3
11	**1182.5425**	591.7749	1164.5320	582.7696	D	290.1459	145.5766	273.1193	137.0633	272.1353	136.5713	2
12					R	175.1190	88.0631	158.0924	79.5498			1

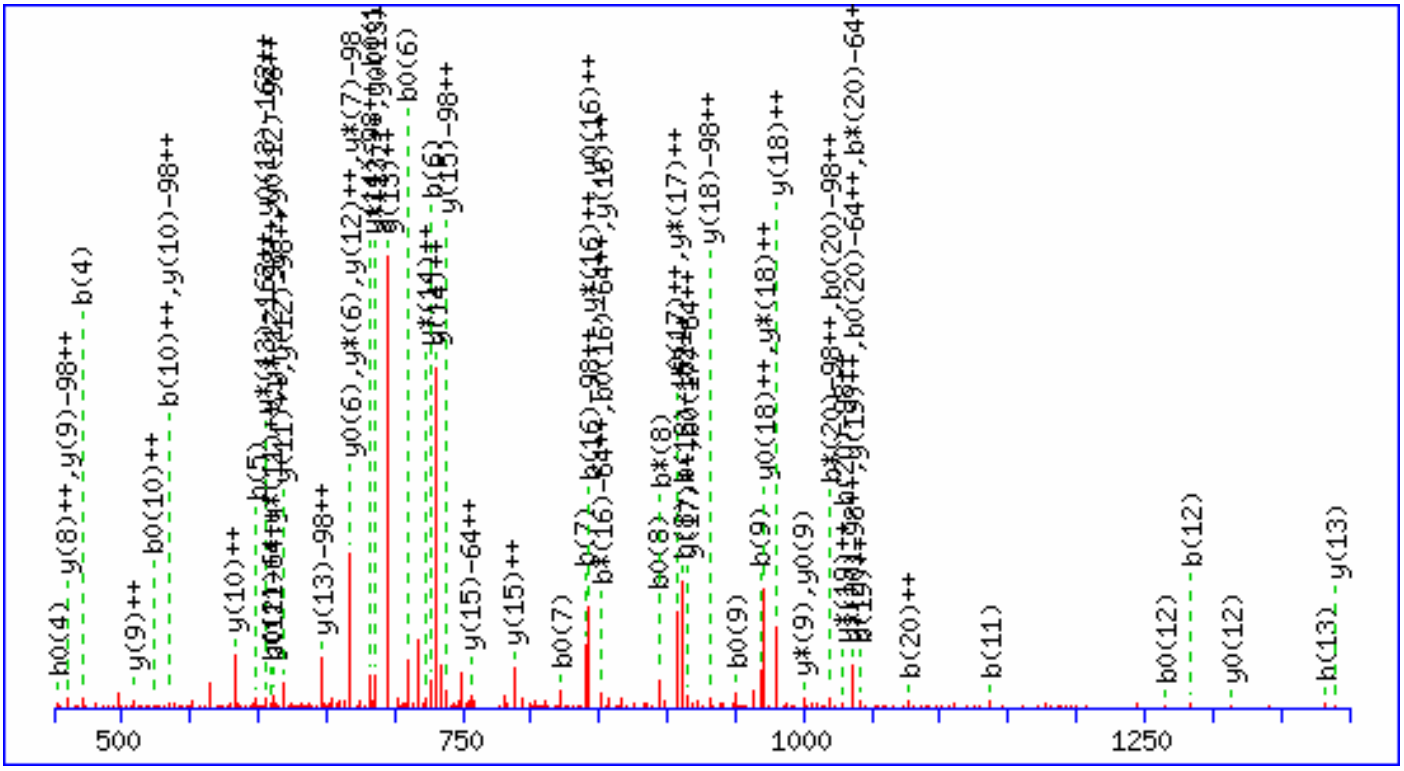
MS/MS Fragmentation of **LYGAEEGVSWIARPVR**



Monoisotopic mass of neutral peptide Mr(calc): 1881.9138
Variable modifications:
S9 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 51 **Expect:** 0.00043
Matches (Bold Red): 37/216 fragment ions using 57 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							16
2	277.1547	139.0810					Y	1671.8601	836.4337	1654.8336	827.9204	1653.8496	827.4284	15
3	334.1761	167.5917					G	1508.7968	**754.9020**	1491.7702	746.3888	1490.7862	745.8968	14
4	405.2132	203.1103					A	1451.7753	726.3913	1434.7488	717.8780	1433.7648	717.3860	13
5	534.2558	267.6316			**516.2453**	258.6263	E	**1380.7382**	690.8727	1363.7117	**682.3595**	1362.7277	**681.8675**	12
6	663.2984	332.1528			645.2879	323.1476	E	**1251.6956**	**626.3515**	1234.6691	617.8382	1233.6851	617.3462	11
7	720.3199	360.6636			702.3093	351.6583	G	**1122.6530**	**561.8302**	1105.6265	553.3169	1104.6425	552.8249	10
8	819.3883	410.1978			801.3777	401.1925	V	1065.6316	533.3194	1048.6050	524.8062	1047.6210	524.3141	9
9	888.4097	444.7085			870.3992	435.7032	S	**966.5632**	483.7852	949.5366	475.2719	948.5526	474.7799	8
10	**1074.4891**	537.7482			1056.4785	528.7429	W	**897.5417**	449.2745	880.5152	440.7612			7
11	**1187.5731**	594.2902			1169.5625	585.2849	I	**711.4624**	356.2348	694.4359	347.7216			6
12	**1258.6102**	629.8088			1240.5997	620.8035	A	**598.3783**	299.6928	581.3518	291.1795			5
13	**1414.7113**	707.8593	1397.6848	699.3460	1396.7008	698.8540	R	**527.3412**	264.1743	510.3147	255.6610			4
14	1511.7641	756.3857	**1494.7375**	747.8724	1493.7535	747.3804	P	**371.2401**	186.1237	**354.2136**	177.6104			3
15	**1610.8325**	805.9199	1593.8060	797.4066	1592.8219	796.9146	V	274.1874	137.5973	257.1608	129.0840			2
16							R	175.1190	88.0631	158.0924	79.5498			1

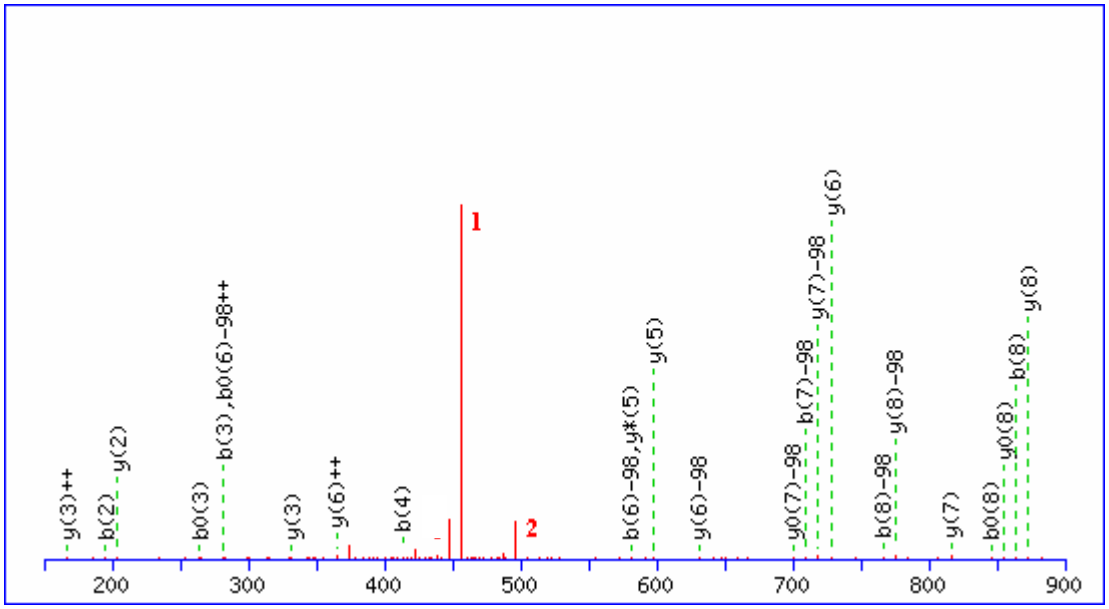
MS/MS Fragmentation of **DLIEQQLAGPAMVHPpSEAVAK**



Monoisotopic mass of neutral peptide Mr(calc): 2299.0919
Variable modifications:
M12 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
S16 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 64 **Expect:** 2.3e-05
Matches (Bold Red): 83/560 fragment ions using 93 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	116.0342	58.5207			98.0237	49.5155	D							21
2	**229.1183**	115.0628			211.1077	106.0575	L	2185.0723	1093.0398	2168.0457	1084.5265	2167.0617	1084.0345	20
3	**342.2023**	171.6048			**324.1918**	162.5995	I	2071.9882	**1036.4977**	2054.9617	**1027.9845**	2053.9777	1027.4925	19
4	**471.2449**	236.1261			**453.2344**	227.1208	E	1958.9042	**979.9557**	1941.8776	**971.4424**	1940.8936	**970.9504**	18
5	**599.3035**	300.1554	582.2770	291.6421	581.2930	291.1501	Q	1829.8616	**915.4344**	1812.8350	**906.9211**	1811.8510	**906.4291**	17
6	**727.3621**	364.1847	710.3355	355.6714	**709.3515**	355.1794	Q	1701.8030	**851.4051**	1684.7764	**842.8919**	1683.7924	**842.3998**	16
7	**840.4462**	420.7267	823.4196	412.2134	**822.4356**	411.7214	L	1573.7444	**787.3758**	1556.7179	778.8626	1555.7338	778.3706	15
8	**911.4833**	456.2453	**894.4567**	447.7320	**893.4727**	447.2400	A	1460.6603	**730.8338**	1443.6338	**722.3205**	1442.6498	721.8285	14
9	**968.5047**	484.7560	951.4782	476.2427	**950.4942**	475.7507	G	**1389.6232**	**695.3153**	1372.5967	**686.8020**	1371.6127	**686.3100**	13
10	1065.5575	**533.2824**	1048.5310	524.7691	1047.5469	**524.2771**	P	1332.6018	**666.8045**	1315.5752	658.2912	**1314.5912**	657.7992	12
11	**1136.5946**	568.8009	1119.5681	560.2877	1118.5841	559.7957	A	1235.5490	**618.2781**	1218.5225	**609.7649**	1217.5384	**609.2729**	11
12	**1283.6300**	642.3186	1266.6035	633.8054	**1265.6195**	633.3134	M	1164.5119	**582.7596**	1147.4853	574.2463	1146.5013	573.7543	10
13	**1382.6984**	691.8529	1365.6719	683.3396	1364.6879	**682.8476**	V	1017.4765	**509.2419**	**1000.4499**	500.7286	**999.4659**	500.2366	9
14	1519.7573	760.3823	1502.7308	751.8690	1501.7468	751.3770	H	918.4081	**459.7077**	901.3815	451.1944	900.3975	450.7024	8
15	1616.8101	808.9087	1599.7836	800.3954	1598.7995	799.9034	P	781.3492	**391.1782**	764.3226	382.6649	763.3386	382.1729	7
16	1783.8085	892.4079	1766.7819	883.8946	1765.7979	883.4026	S	684.2964	**342.6518**	**667.2698**	334.1386	**666.2858**	333.6466	6
17	1912.8511	956.9292	1895.8245	948.4159	1894.8405	947.9239	E	517.2980	259.1527	500.2715	250.6394	499.2875	250.1474	5
18	1983.8882	992.4477	1966.8616	983.9344	1965.8776	983.4424	A	388.2554	194.6314	**371.2289**	186.1181			4
19	2082.9566	**1041.9819**	2065.9300	1033.4687	2064.9460	1032.9766	V	317.2183	159.1128	300.1918	150.5995			3
20	2153.9937	**1077.5005**	2136.9671	1068.9872	2135.9831	1068.4952	A	218.1499	109.5786	201.1234	101.0653			2
21							K	147.1128	74.0600	130.0863	65.5468			1

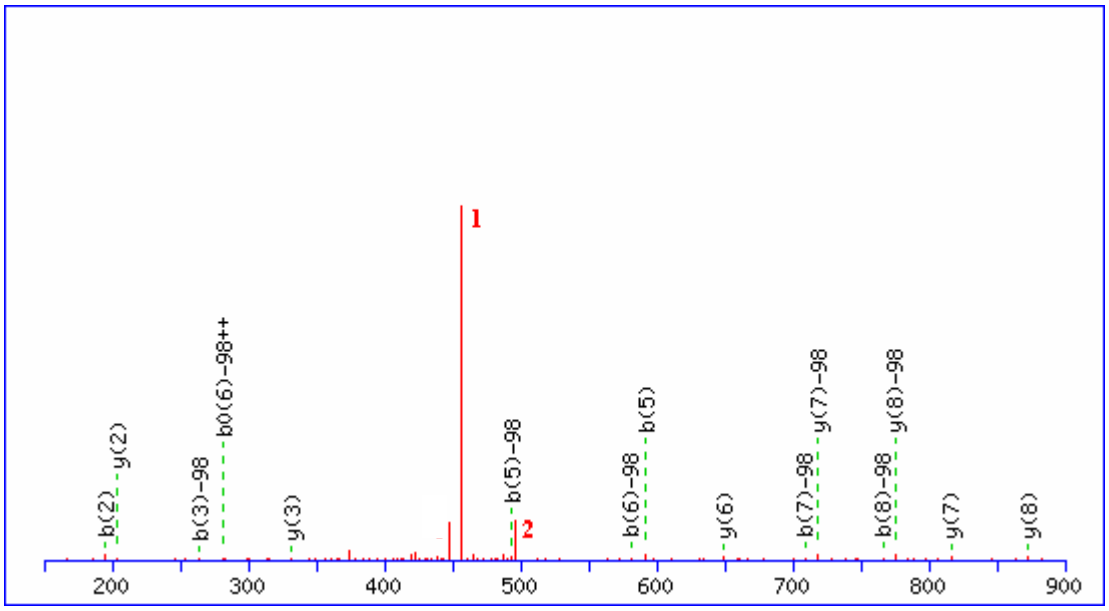
MS/MS Fragmentation of **HGSMVpSQGK**



Monoisotopic mass of neutral peptide Mr(calc): 1009.4052
Variable modifications:
S6 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 36 **Expect:** 0.0047
Matches (Bold Red): 24/120 fragment ions using 45 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	138.0662	69.5367					H							9
2	**195.0876**	98.0475					G	**775.3767**	388.1920	758.3501	379.6787	757.3661	379.1867	8
3	**282.1197**	141.5635			**264.1091**	132.5582	S	**718.3552**	359.6812	701.3287	351.1680	**700.3447**	350.6760	7
4	**413.1602**	207.0837			395.1496	198.0784	M	**631.3232**	316.1652	614.2966	307.6520	613.3126	307.1600	6
5	512.2286	256.6179			494.2180	247.6126	V	500.2827	250.6450	483.2562	242.1317	482.2721	241.6397	5
6	**581.2500**	291.1286			563.2395	**282.1234**	S	401.2143	201.1108	384.1877	192.5975	383.2037	192.1055	4
7	**709.3086**	355.1579	692.2821	346.6447	691.2980	346.1527	Q	**332.1928**	**166.6001**	315.1663	158.0868			3
8	**766.3301**	383.6687	749.3035	375.1554	748.3195	374.6634	G	**204.1343**	102.5708	187.1077	94.0575			2
9							K	147.1128	74.0600	130.0863	65.5468			1

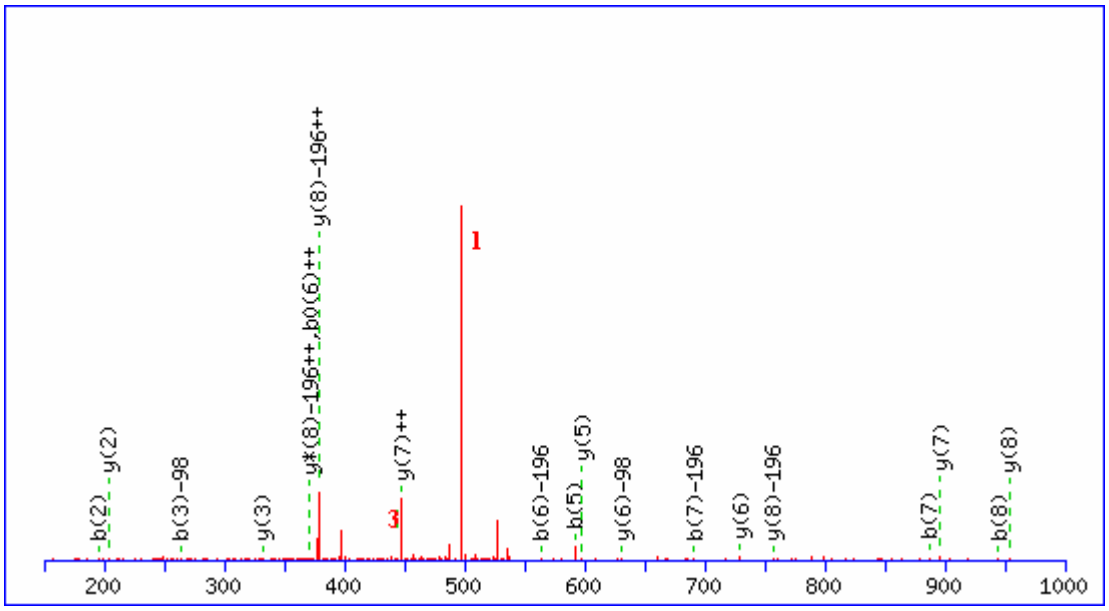
MS/MS Fragmentation of **HGp*SMVSQGK**



Monoisotopic mass of neutral peptide Mr(calc): 1009.4052
Variable modifications:
S3 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 49 **Expect:** 0.00025
Matches (Bold Red): 15/114 fragment ions using 23 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	138.0662	69.5367					H							9
2	**195.0876**	98.0475					G	**775.3767**	388.1920	758.3501	379.6787	757.3661	379.1867	8
3	**264.1091**	132.5582			246.0985	123.5529	S	**718.3552**	359.6812	701.3287	351.1680	700.3447	350.6760	7
4	395.1496	198.0784			377.1390	189.0731	M	**649.3338**	325.1705	632.3072	316.6572	631.3232	316.1652	6
5	**494.2180**	247.6126			476.2074	238.6074	V	518.2933	259.6503	501.2667	251.1370	500.2827	250.6450	5
6	**581.2500**	291.1286			563.2395	**282.1234**	S	419.2249	210.1161	402.1983	201.6028	401.2143	201.1108	4
7	**709.3086**	355.1579	692.2821	346.6447	691.2980	346.1527	Q	**332.1928**	166.6001	315.1663	158.0868			3
8	**766.3301**	383.6687	749.3035	375.1554	748.3195	374.6634	G	**204.1343**	102.5708	187.1077	94.0575			2
9							K	147.1128	74.0600	130.0863	65.5468			1

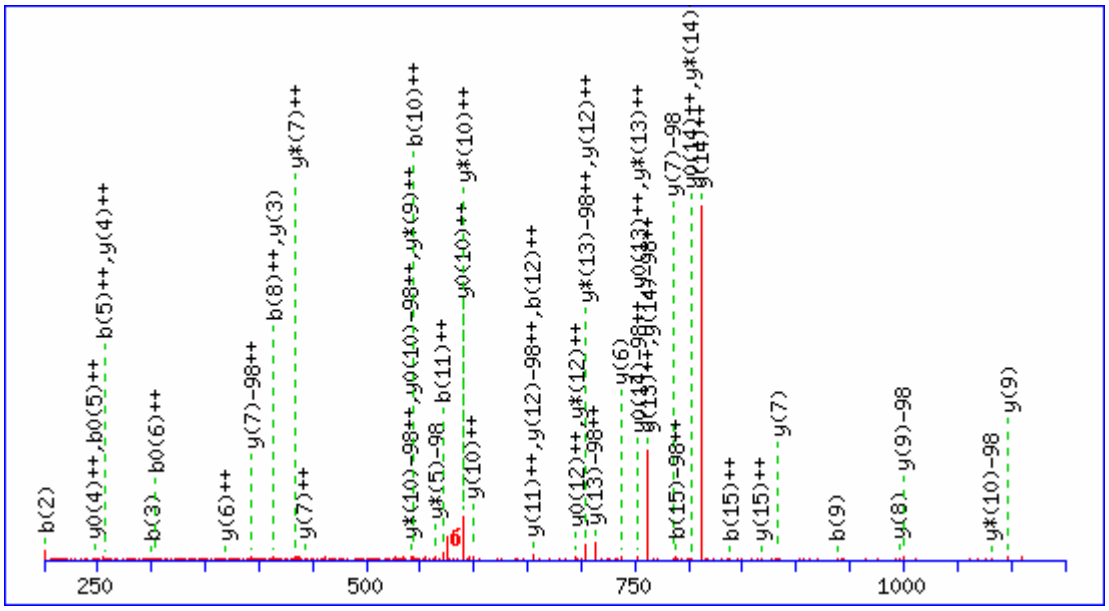
MS/MS Fragmentation of **HGpSMVpSQGK**



Monoisotopic mass of neutral peptide Mr(calc): 1089.3716
Variable modifications:
S3 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
S6 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 30 **Expect:** 0.0098
Matches (Bold Red): 19/132 fragment ions using 44 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	138.0662	69.5367					H							9
2	**195.0877**	98.0475					G	**953.3199**	477.1636	936.2934	468.6503	935.3093	468.1583	8
3	362.0860	181.5466			344.0754	172.5414	S	**896.2984**	**448.6529**	879.2719	440.1396	878.2879	439.6476	7
4	493.1265	247.0669			475.1159	238.0616	M	**729.3001**	365.1537	712.2735	356.6404	711.2895	356.1484	6
5	**592.1949**	296.6011			574.1843	287.5958	V	**598.2596**	299.6334	581.2331	291.1202	580.2490	290.6282	5
6	759.1933	380.1003			741.1827	**371.0950**	S	499.1912	250.0992	482.1646	241.5860	481.1806	241.0940	4
7	**887.2518**	444.1296	870.2253	435.6163	869.2413	435.1243	Q	**332.1928**	166.6001	315.1663	158.0868			3
8	**944.2733**	472.6403	927.2467	464.1270	926.2627	463.6350	G	**204.1343**	102.5708	187.1077	94.0575			2
9							K	147.1128	74.0600	130.0863	65.5468			1

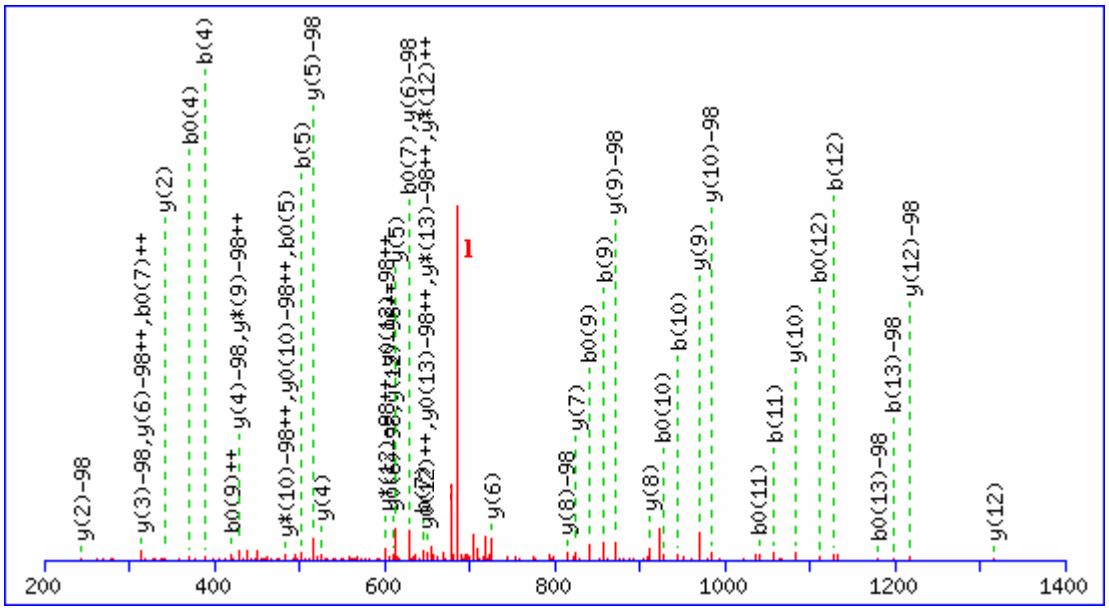
MS/MS Fragmentation of **SLVDPLVTLFGpSVHEK**



Monoisotopic mass of neutral peptide Mr(calc): 1819.9121
Variable modifications:
S12 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 39 **Expect:** 0.0071
Matches (Bold Red): 49/230 fragment ions using 72 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	88.0393	44.5233	70.0287	35.5180	S							16
2	**201.1234**	101.0653	183.1128	92.0600	L	1733.8874	**867.4473**	1716.8608	858.9340	1715.8768	858.4420	15
3	**300.1918**	150.5995	282.1812	141.5942	V	1620.8033	**810.9053**	1603.7767	**802.3920**	1602.7927	**801.9000**	14
4	415.2187	208.1130	397.2082	199.1077	D	1521.7349	**761.3711**	1504.7083	**752.8578**	1503.7243	**752.3658**	13
5	512.2715	**256.6394**	494.2609	**247.6341**	P	1406.7079	**703.8576**	1389.6814	**695.3443**	1388.6974	**694.8523**	12
6	625.3556	313.1814	607.3450	**304.1761**	L	1309.6552	**655.3312**	1292.6286	646.8180	1291.6446	646.3259	11
7	724.4240	362.7156	706.4134	353.7103	V	1196.5711	**598.7892**	1179.5446	**590.2759**	1178.5605	**589.7839**	10
8	825.4716	**413.2395**	807.4611	404.2342	T	**1097.5027**	549.2550	1080.4761	**540.7417**	1079.4921	540.2497	9
9	**938.5557**	469.7815	920.5451	460.7762	L	**996.4550**	498.7311	979.4285	490.2179	978.4445	489.7259	8
10	1085.6241	**543.3157**	1067.6136	534.3104	F	**883.3710**	**442.1891**	866.3444	**433.6758**	865.3604	433.1838	7
11	1142.6456	**571.8264**	1124.6350	562.8211	G	**736.3025**	**368.6549**	719.2760	360.1416	718.2920	359.6496	6
12	1309.6439	**655.3256**	1291.6334	646.3203	S	679.2811	340.1442	662.2545	331.6309	661.2705	331.1389	5
13	1408.7124	704.8598	1390.7018	695.8545	V	512.2827	**256.6450**	495.2562	248.1317	494.2722	**247.6397**	4
14	1545.7713	773.3893	1527.7607	764.3840	H	**413.2143**	207.1108	396.1878	198.5975	395.2037	198.1055	3
15	1674.8139	**837.9106**	1656.8033	828.9053	E	276.1554	138.5813	259.1288	130.0681	258.1448	129.5761	2
16					K	147.1128	74.0600	130.0863	65.5468			1

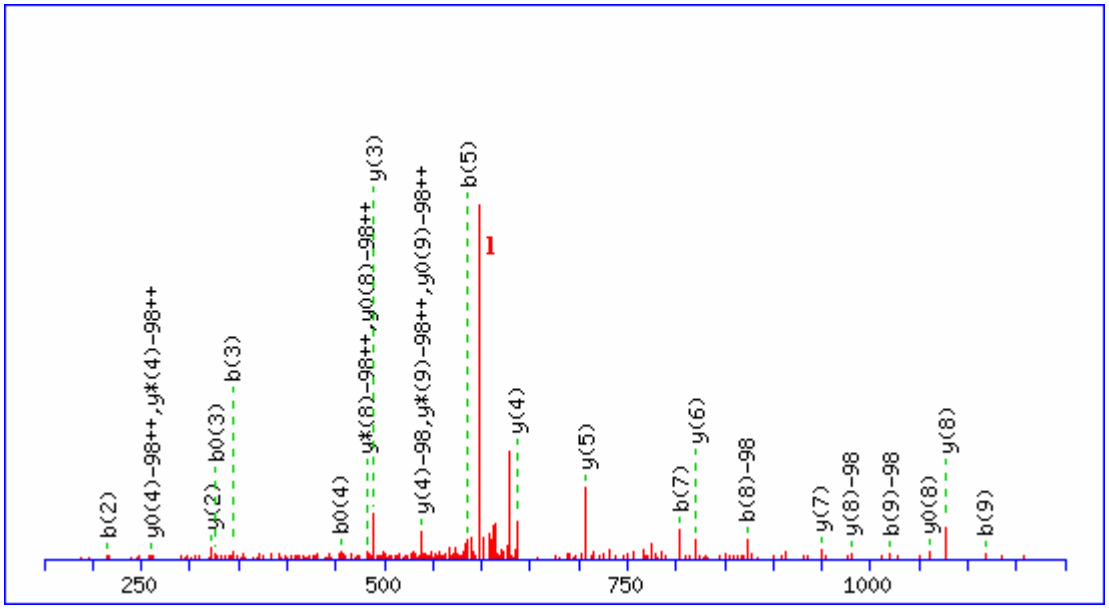
MS/MS Fragmentation of **GPSMLGSVLSLApSR**



Monoisotopic mass of neutral peptide Mr(calc): 1469.6949
Variable modifications:
M4 : Oxidation (M)
S13 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 79 **Expect:** 5.5e-07
Matches (Bold Red): 48/200 fragment ions using 53 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180			G							14
2	155.0815	78.0444			P	1315.7038	658.3555	1298.6773	**649.8423**	1297.6933	**649.3503**	13
3	242.1135	121.5604	224.1030	112.5551	S	**1218.6511**	**609.8292**	1201.6245	**601.3159**	1200.6405	**600.8239**	12
4	**389.1489**	195.0781	**371.1384**	186.0728	M	1131.6190	566.3132	1114.5925	557.7999	1113.6085	557.3079	11
5	**502.2330**	251.6201	**484.2224**	242.6148	L	**984.5836**	492.7955	967.5571	**484.2822**	966.5731	**483.7902**	10
6	559.2544	280.1309	541.2439	271.1256	G	**871.4996**	436.2534	854.4730	**427.7401**	853.4890	427.2481	9
7	**646.2865**	323.6469	**628.2759**	**314.6416**	S	**814.4781**	407.7427	797.4516	399.2294	796.4675	398.7374	8
8	745.3549	373.1811	727.3443	364.1758	V	727.4461	364.2267	710.4195	355.7134	709.4355	355.2214	7
9	**858.4389**	429.7231	**840.4284**	**420.7178**	L	**628.3777**	**314.6925**	611.3511	306.1792	**610.3671**	305.6872	6
10	**945.4710**	473.2391	**927.4604**	464.2338	S	**515.2936**	258.1504	498.2671	249.6372	497.2830	249.1452	5
11	**1058.5550**	529.7812	**1040.5445**	520.7759	L	**428.2616**	214.6344	411.2350	206.1212	410.2510	205.6291	4
12	**1129.5921**	565.2997	**1111.5816**	556.2944	A	**315.1775**	158.0924	298.1510	149.5791	297.1670	149.0871	3
13	**1198.6136**	599.8104	**1180.6030**	590.8052	S	**244.1404**	122.5738	227.1139	114.0606	226.1298	113.5686	2
14					R	175.1190	88.0631	158.0924	79.5498			1

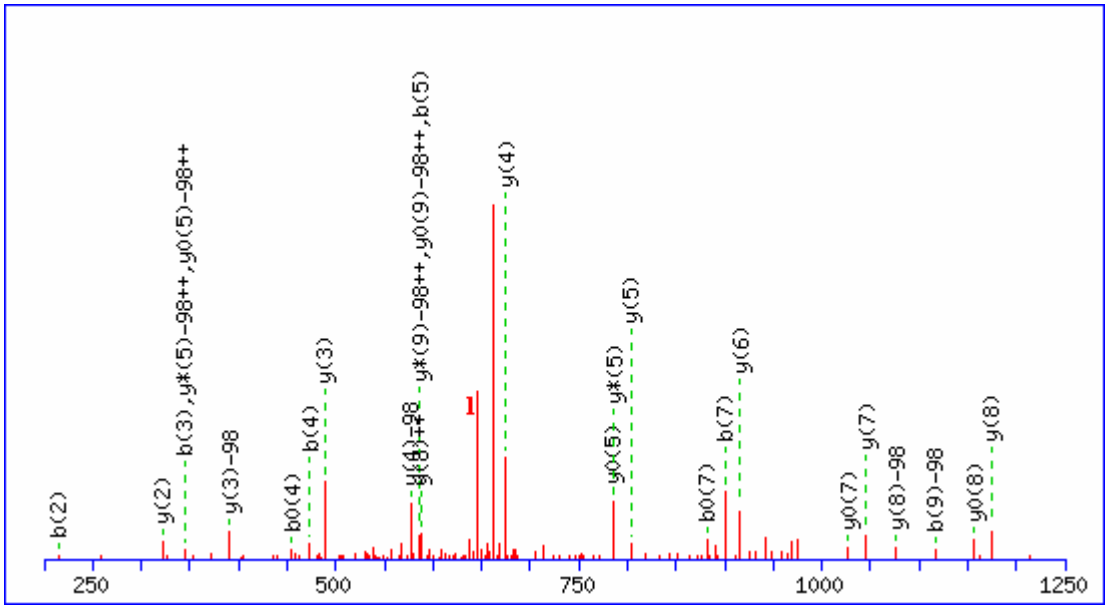
MS/MS Fragmentation of **TLEEIAFpSFR**



Monoisotopic mass of neutral peptide Mr(calc): 1291.5849
Variable modifications:
S8 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 50 **Expect:** 0.00053
Matches (Bold Red): 25/136 fragment ions using 31 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	102.0550	51.5311	84.0444	42.5258	T							10
2	**215.1390**	108.0731	197.1285	99.0679	L	1191.5446	596.2759	1174.5180	587.7626	1173.5340	587.2706	9
3	**344.1816**	172.5944	**326.1710**	163.5892	E	**1078.4605**	539.7339	1061.4339	531.2206	**1060.4499**	530.7286	8
4	473.2242	237.1157	**455.2136**	228.1105	E	**949.4179**	475.2126	932.3914	466.6993	931.4073	466.2073	7
5	**586.3083**	293.6578	568.2977	284.6525	I	**820.3753**	410.6913	803.3488	402.1780	802.3647	401.6860	6
6	657.3454	329.1763	639.3348	320.1710	A	**707.2912**	354.1493	690.2647	345.6360	689.2807	345.1440	5
7	**804.4138**	402.7105	786.4032	393.7053	F	**636.2541**	318.6307	619.2276	310.1174	618.2436	309.6254	4
8	971.4121	486.2097	953.4016	477.2044	S	**489.1857**	245.0965	472.1592	236.5832	471.1752	236.0912	3
9	**1118.4806**	559.7439	1100.4700	550.7386	F	**322.1874**	161.5973	305.1608	153.0840			2
10					R	175.1190	88.0631	158.0924	79.5498			1

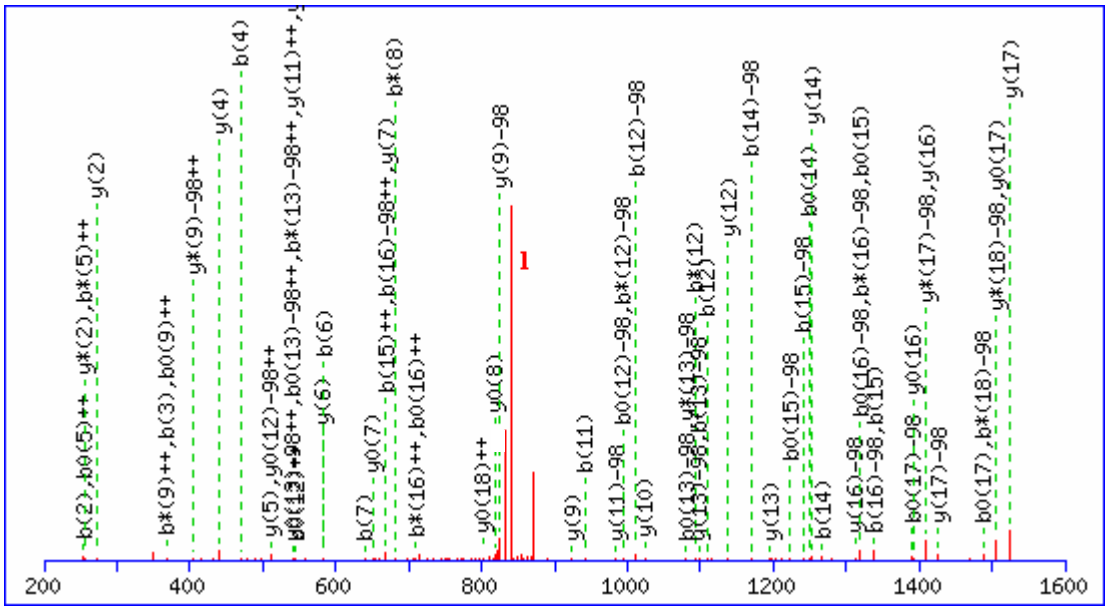
MS/MS Fragmentation of **TLEEIQWpSFR**



Monoisotopic mass of neutral peptide Mr(calc): 1387.6173
Variable modifications:
S8 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 53 **Expect:** 0.00036
Matches (Bold Red): 27/148 fragment ions using 29 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	102.0550	51.5311			84.0444	42.5258	T							10
2	**215.1390**	108.0731			197.1285	99.0679	L	1287.5769	644.2921	1270.5504	635.7788	1269.5664	635.2868	9
3	**344.1816**	172.5944			326.1710	163.5892	E	**1174.4929**	**587.7501**	1157.4663	579.2368	**1156.4823**	578.7448	8
4	**473.2242**	237.1157			**455.2136**	228.1105	E	**1045.4503**	523.2288	1028.4237	514.7155	**1027.4397**	514.2235	7
5	**586.3083**	293.6578			568.2977	284.6525	I	**916.4077**	458.7075	899.3811	450.1942	898.3971	449.7022	6
6	714.3668	357.6871	697.3403	349.1738	696.3563	348.6818	Q	**803.3236**	402.1654	**786.2971**	393.6522	**785.3130**	393.1602	5
7	**900.4462**	450.7267	883.4196	442.2134	**882.4356**	441.7214	W	**675.2650**	338.1362	658.2385	329.6229	657.2545	329.1309	4
8	1067.4445	534.2259	1050.4180	525.7126	1049.4339	525.2206	S	**489.1857**	245.0965	472.1592	236.5832	471.1752	236.0912	3
9	1214.5129	607.7601	1197.4864	599.2468	1196.5024	598.7548	F	**322.1874**	161.5973	305.1608	153.0840			2
10							R	175.1190	88.0631	158.0924	79.5498			1

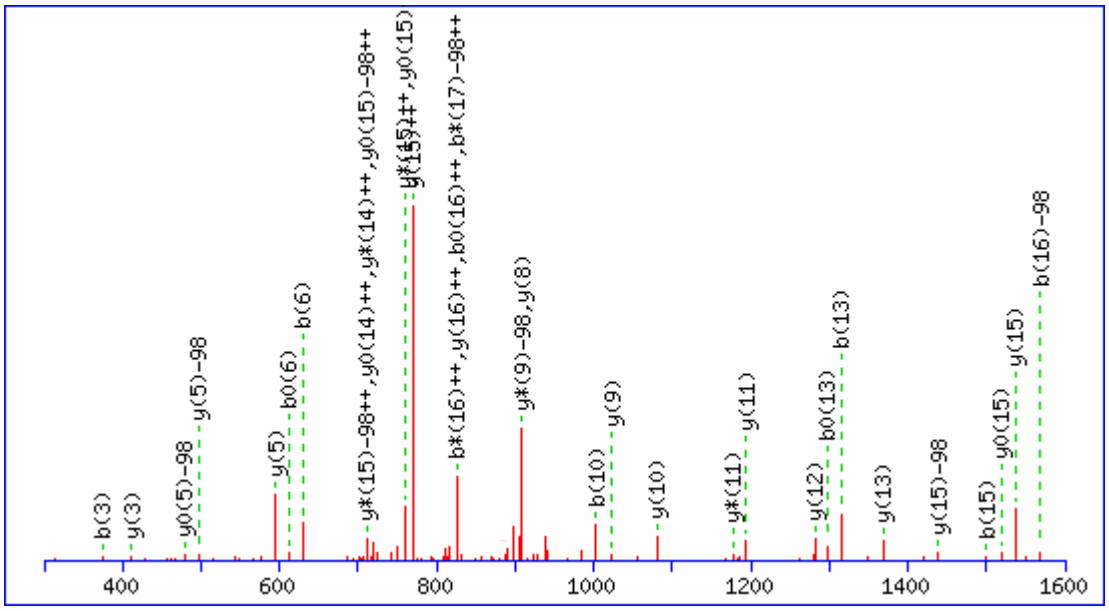
MS/MS Fragmentation of **RPNTGGGGGTSp*SSAAPAPR**



Monoisotopic mass of neutral peptide Mr(calc): 1776.7904
Variable modifications:
S12 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 75 **Expect:** 1.7e-06
Matches (Bold Red): 71/306 fragment ions using 77 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	157.1084	79.0578	140.0818	70.5446			R							19
2	**254.1611**	127.5842	237.1346	119.0709			P	1523.7197	762.3635	**1506.6931**	753.8502	1505.7091	753.3582	18
3	**368.2041**	184.6057	351.1775	176.0924			N	**1426.6669**	713.8371	**1409.6404**	705.3238	1408.6563	704.8318	17
4	**469.2518**	235.1295	452.2252	226.6162	451.2412	226.1242	T	**1312.6240**	656.8156	1295.5974	648.3024	1294.6134	647.8103	16
5	526.2732	263.6402	509.2467	**255.1270**	508.2627	**254.6350**	G	1211.5763	606.2918	1194.5498	597.7785	1193.5657	597.2865	15
6	**583.2947**	292.1510	566.2681	283.6377	565.2841	283.1457	G	1154.5548	577.7811	1137.5283	569.2678	1136.5443	568.7758	14
7	**640.3161**	320.6617	623.2896	312.1484	622.3056	311.6564	G	**1097.5334**	549.2703	**1080.5068**	**540.7571**	1079.5228	**540.2650**	13
8	697.3376	349.1724	**680.3110**	340.6592	679.3270	340.1672	G	1040.5119	520.7596	1023.4854	512.2463	1022.5014	**511.7543**	12
9	754.3591	377.6832	737.3325	**369.1699**	736.3485	**368.6779**	G	**983.4905**	492.2489	966.4639	483.7356	965.4799	483.2436	11
10	855.4067	428.2070	838.3802	419.6937	837.3962	419.2017	T	926.4690	463.7381	909.4425	455.2249	908.4584	454.7329	10
11	**942.4388**	471.7230	925.4122	463.2097	924.4282	462.7177	S	**825.4213**	413.2143	808.3948	**404.7010**	807.4108	404.2090	9
12	**1011.4602**	506.2338	**994.4337**	497.7205	**993.4497**	497.2285	S	738.3893	369.6983	721.3627	361.1850	720.3787	360.6930	8
13	**1098.4923**	549.7498	1081.4657	**541.2365**	**1080.4817**	**540.7445**	S	**669.3678**	335.1876	652.3413	326.6743	**651.3573**	326.1823	7
14	**1169.5294**	585.2683	1152.5028	576.7550	1151.5188	576.2630	A	**582.3358**	291.6715	565.3093	283.1583			6
15	**1240.5665**	620.7869	1223.5399	612.2736	**1222.5559**	611.7816	A	**511.2987**	256.1530	494.2721	247.6397			5
16	**1337.6192**	**669.3133**	**1320.5927**	660.8000	**1319.6087**	660.3080	P	**440.2616**	220.6344	423.2350	212.1212			4
17	1408.6563	704.8318	1391.6298	696.3185	**1390.6458**	695.8265	A	343.2088	172.1080	326.1823	163.5948			3
18	1505.7091	753.3582	**1488.6826**	744.8449	1487.6985	744.3529	P	**272.1717**	136.5895	**255.1452**	128.0762			2
19							R	175.1190	88.0631	158.0924	79.5498			1

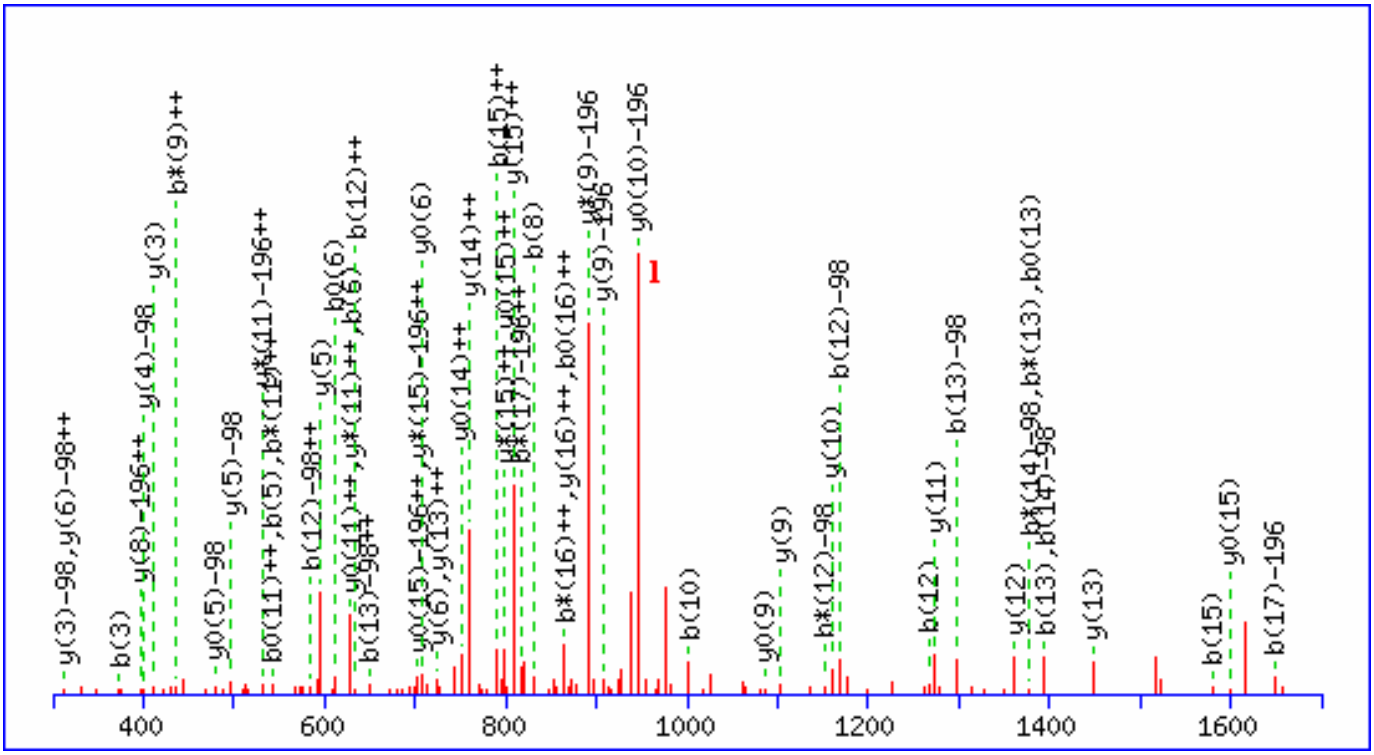
MS/MS Fragmentation of **FLLPASSNGDPSEPSp*SPK**



Monoisotopic mass of neutral peptide Mr(calc): 1908.8506
Variable modifications:
S16 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 87 **Expect:** 1.1e-07
Matches (Bold Red): 34/278 fragment ions using 27 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	148.0757	74.5415					F							18
2	261.1598	131.0835					L	1762.7895	881.8984	1745.7629	873.3851	1744.7789	872.8931	17
3	**374.2438**	187.6255					L	1649.7054	**825.3564**	1632.6789	816.8431	1631.6949	816.3511	16
4	471.2966	236.1519					P	**1536.6214**	**768.8143**	1519.5948	**760.3010**	**1518.6108**	**759.8090**	15
5	542.3337	271.6705					A	1439.5686	720.2879	1422.5421	**711.7747**	1421.5580	**711.2827**	14
6	**629.3657**	315.1865			**611.3552**	306.1812	S	**1368.5315**	684.7694	1351.5049	676.2561	1350.5209	675.7641	13
7	716.3978	358.7025			698.3872	349.6972	S	**1281.4995**	641.2534	1264.4729	632.7401	1263.4889	632.2481	12
8	830.4407	415.7240	813.4141	407.2107	812.4301	406.7187	N	**1194.4674**	597.7374	**1177.4409**	589.2241	1176.4569	588.7321	11
9	887.4621	444.2347	870.4356	435.7214	869.4516	435.2294	G	**1080.4245**	540.7159	1063.3980	532.2026	1062.4139	531.7106	10
10	**1002.4891**	501.7482	985.4625	493.2349	984.4785	492.7429	D	**1023.4030**	512.2052	1006.3765	503.6919	1005.3925	503.1999	9
11	1099.5418	550.2746	1082.5153	541.7613	1081.5313	541.2693	P	**908.3761**	454.6917	891.3495	446.1784	890.3655	445.6864	8
12	1186.5739	593.7906	1169.5473	585.2773	1168.5633	584.7853	S	811.3233	406.1653	794.2968	397.6520	793.3128	397.1600	7
13	**1315.6165**	658.3119	1298.5899	649.7986	**1297.6059**	649.3066	E	724.2913	362.6493	707.2648	354.1360	706.2807	353.6440	6
14	1412.6692	706.8383	1395.6427	698.3250	1394.6587	697.8330	P	**595.2487**	298.1280	578.2222	289.6147	577.2381	289.1227	5
15	**1499.7013**	750.3543	1482.6747	741.8410	1481.6907	741.3490	S	498.1959	249.6016	481.1694	241.0883	480.1854	240.5963	4
16	1666.6996	833.8534	1649.6731	**825.3402**	1648.6891	**824.8482**	S	**411.1639**	206.0856	394.1374	197.5723	393.1534	197.0803	3
17	1763.7524	882.3798	1746.7258	873.8666	1745.7418	873.3745	P	244.1656	122.5864	227.1390	114.0731			2
18							K	147.1128	74.0600	130.0863	65.5468			1

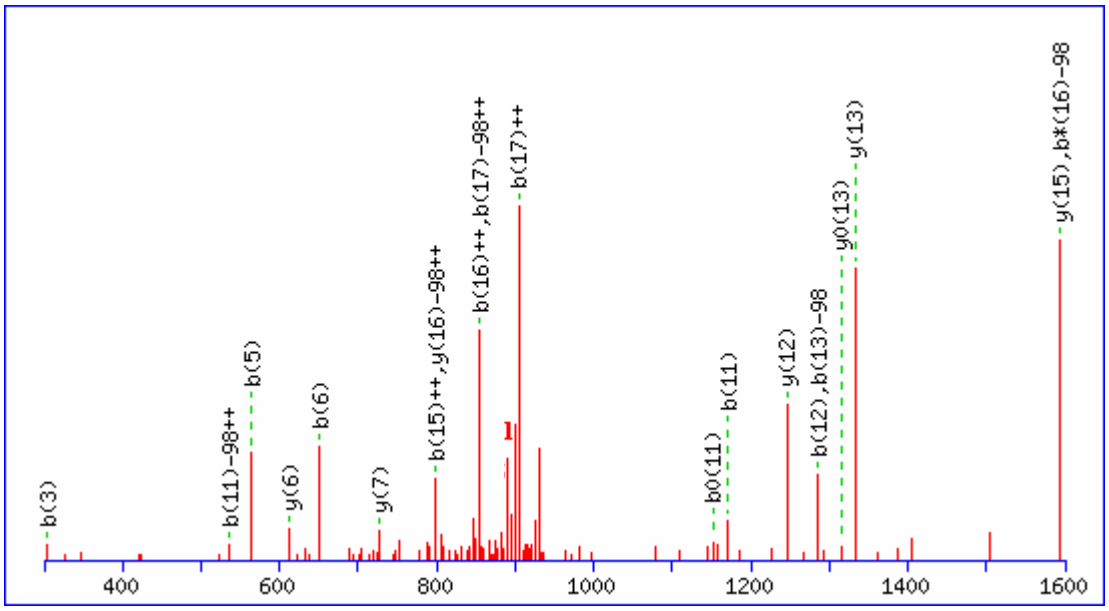
MS/MS Fragmentation of **FLLPASSNGDPpSEPSp*SPK**



Monoisotopic mass of neutral peptide Mr(calc): 1988.8169
Variable modifications:
S12 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
S16 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 31 **Expect:** 0.043
Matches (Bold Red): 59/302 fragment ions using 90 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	148.0757	74.5415					F							18
2	261.1597	131.0835					L	1646.8020	823.9046	1629.7754	815.3914	1628.7914	814.8994	17
3	**374.2438**	187.6255					L	1533.7179	767.3626	1516.6914	758.8493	1515.7074	758.3573	16
4	471.2966	236.1519					P	1420.6339	710.8206	1403.6073	**702.3073**	1402.6233	**701.8153**	15
5	**542.3337**	271.6705					A	1323.5811	662.2942	1306.5546	653.7809	1305.5706	653.2889	14
6	**629.3657**	315.1865			**611.3551**	306.1812	S	1252.5440	626.7756	1235.5175	618.2624	1234.5334	617.7704	13
7	716.3977	358.7025			698.3872	349.6972	S	1165.5120	583.2596	1148.4854	574.7464	1147.5014	574.2543	12
8	**830.4407**	415.7240	813.4141	407.2107	812.4301	406.7187	N	1078.4799	539.7436	1061.4534	**531.2303**	1060.4694	530.7383	11
9	887.4621	444.2347	870.4356	**435.7214**	869.4516	435.2294	G	964.4370	482.7221	947.4105	474.2089	**946.4265**	473.7169	10
10	**1002.4891**	501.7482	985.4625	493.2349	984.4785	492.7429	D	**907.4156**	454.2114	**890.3890**	445.6981	889.4050	445.2061	9
11	1099.5418	550.2746	1082.5153	**541.7613**	1081.5313	**541.2693**	P	792.3886	**396.6979**	775.3621	388.1847	774.3781	387.6927	8
12	**1168.5633**	**584.7853**	**1151.5367**	576.2720	1150.5527	575.7800	S	695.3359	348.1716	678.3093	339.6583	677.3253	339.1663	7
13	**1297.6059**	**649.3066**	1280.5793	640.7933	1279.5953	640.3013	E	626.3144	**313.6608**	609.2879	305.1476	608.3038	304.6556	6
14	**1394.6586**	697.8330	**1377.6321**	689.3197	1376.6481	688.8277	P	**497.2718**	249.1395	480.2453	240.6263	**479.2612**	240.1343	5
15	1481.6907	741.3490	1464.6641	732.8357	1463.6801	732.3437	S	**400.2190**	200.6132	383.1925	192.0999	382.2085	191.6079	4
16	1550.7121	775.8597	1533.6856	767.3464	1532.7016	766.8544	S	**313.1870**	157.0971	296.1605	148.5839	295.1765	148.0919	3
17	**1647.7649**	824.3861	1630.7383	**815.8728**	1629.7543	815.3808	P	244.1656	122.5864	227.1390	114.0731			2
18							K	147.1128	74.0600	130.0863	65.5468			1

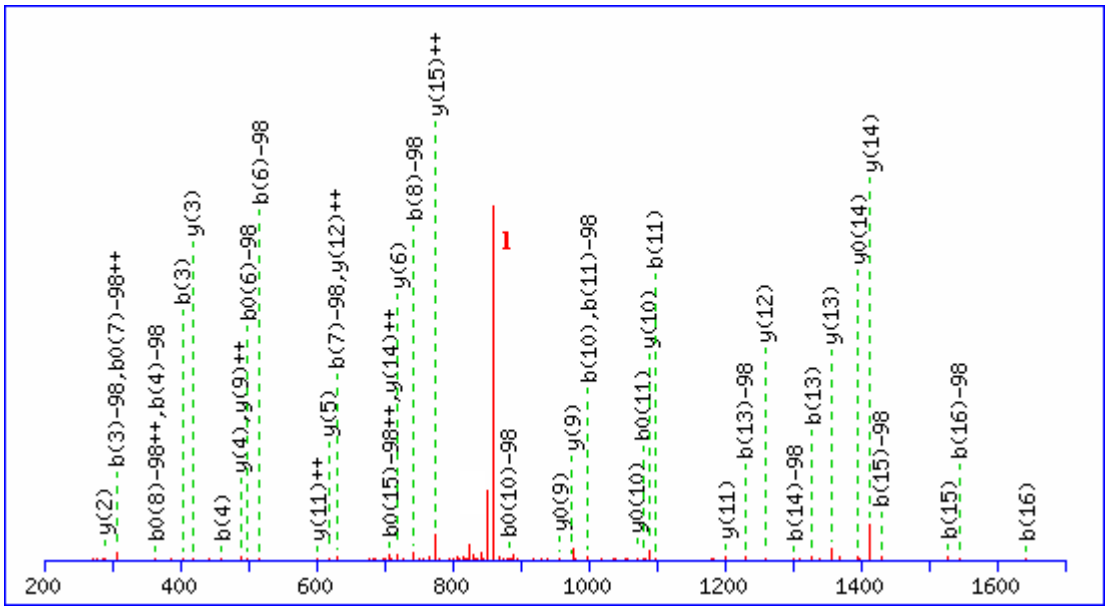
MS/MS Fragmentation of **GFVPYSPGpSPTDPNVIVA**



Monoisotopic mass of neutral peptide Mr(calc): 1895.8707
Variable modifications:
S9 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 34 **Expect:** 0.021
Matches (Bold Red): 20/240 fragment ions using 28 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180					G							18
2	205.0972	103.0522					F	1839.8565	920.4319	1822.8299	911.9186	1821.8459	911.4266	17
3	**304.1656**	152.5864					V	1692.7880	846.8977	1675.7615	838.3844	1674.7775	837.8924	16
4	401.2183	201.1128					P	**1593.7196**	797.3635	1576.6931	788.8502	1575.7091	788.3582	15
5	**564.2817**	282.6445					Y	1496.6669	748.8371	1479.6403	740.3238	1478.6563	739.8318	14
6	**651.3137**	326.1605			633.3031	317.1552	S	**1333.6035**	667.3054	1316.5770	658.7921	**1315.5930**	658.3001	13
7	748.3665	374.6869			730.3559	365.6816	P	**1246.5715**	623.7894	1229.5450	615.2761	1228.5609	614.7841	12
8	805.3879	403.1976			787.3774	394.1923	G	1149.5187	575.2630	1132.4922	566.7497	1131.5082	566.2577	11
9	972.3863	486.6968			954.3757	477.6915	S	1092.4973	546.7523	1075.4707	538.2390	1074.4867	537.7470	10
10	1069.4390	535.2232			1051.4285	526.2179	P	925.4989	463.2531	908.4724	454.7398	907.4884	454.2478	9
11	**1170.4867**	585.7470			**1152.4761**	576.7417	T	828.4462	414.7267	811.4196	406.2134	810.4356	405.7214	8
12	**1285.5137**	643.2605			1267.5031	634.2552	D	**727.3985**	364.2029	710.3719	355.6896	709.3879	355.1976	7
13	1382.5664	691.7868			1364.5559	682.7816	P	**612.3715**	306.6894	595.3450	298.1761			6
14	1496.6093	748.8083	1479.5828	740.2950	1478.5988	739.8030	N	515.3188	258.1630	498.2922	249.6498			5
15	1595.6778	**798.3425**	1578.6512	789.8292	1577.6672	789.3372	V	401.2758	201.1416					4
16	1708.7618	**854.8845**	1691.7353	846.3713	1690.7513	845.8793	I	302.2074	151.6074					3
17	1807.8302	**904.4188**	1790.8037	895.9055	1789.8197	895.4135	V	189.1234	95.0653					2
18							A	90.0550	45.5311					1

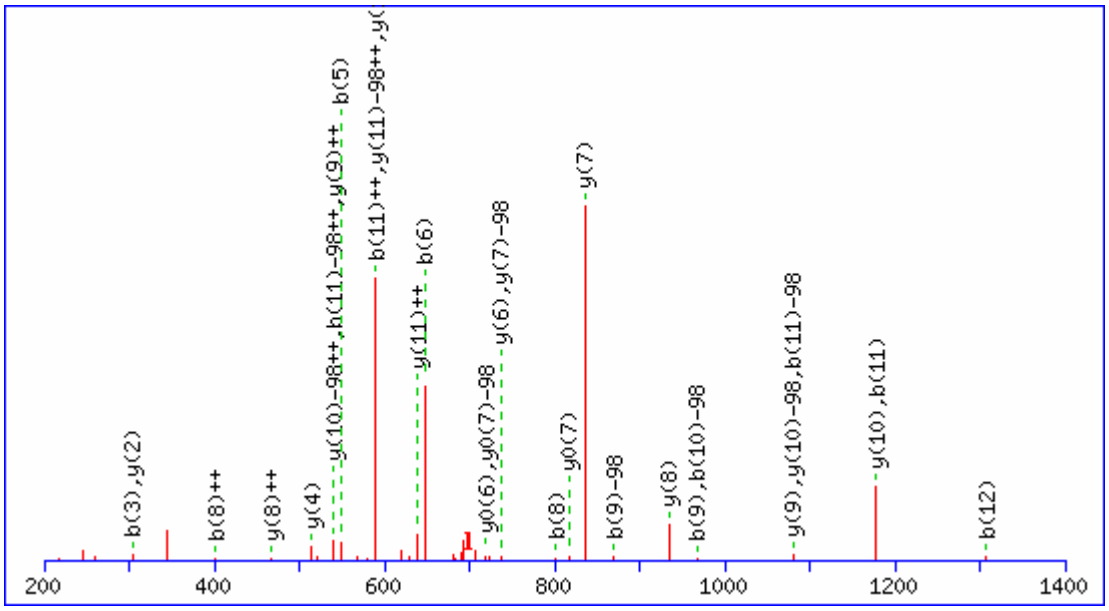
MS/MS Fragmentation of **pSVHGPGLLGTVTEAENR**



Monoisotopic mass of neutral peptide Mr(calc): 1815.8516
Variable modifications:
S1 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 107 **Expect:** 1.3e-09
Matches (Bold Red): 42/224 fragment ions using 41 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	70.0287	35.5180			52.0182	26.5127	S							17
2	169.0971	85.0522			151.0866	76.0469	V	1649.8605	825.4339	1632.8340	816.9206	1631.8500	816.4286	16
3	**306.1561**	153.5817			288.1455	144.5764	H	1550.7921	**775.8997**	1533.7656	767.3864	1532.7815	766.8944	15
4	**363.1775**	182.0924			345.1669	173.0871	G	**1413.7332**	**707.3702**	1396.7067	698.8570	**1395.7226**	698.3650	14
5	460.2303	230.6188			442.2197	221.6135	P	**1356.7117**	678.8595	1339.6852	670.3462	1338.7012	669.8542	13
6	**517.2517**	259.1295			**499.2412**	250.1242	G	**1259.6590**	**630.3331**	1242.6324	621.8199	1241.6484	621.3278	12
7	**630.3358**	315.6715			612.3252	**306.6663**	L	**1202.6375**	**601.8224**	1185.6110	593.3091	1184.6270	592.8171	11
8	**743.4199**	372.2136			725.4093	**363.2083**	L	**1089.5535**	545.2804	1072.5269	536.7671	**1071.5429**	536.2751	10
9	800.4413	400.7243			782.4307	391.7190	G	**976.4694**	**488.7383**	959.4429	480.2251	**958.4588**	479.7331	9
10	901.4890	451.2481			**883.4784**	442.2429	T	919.4479	460.2276	902.4214	451.7143	901.4374	451.2223	8
11	**1000.5574**	500.7823			982.5468	491.7771	V	818.4003	409.7038	801.3737	401.1905	800.3897	400.6985	7
12	1101.6051	551.3062			1083.5945	542.3009	T	**719.3319**	360.1696	702.3053	351.6563	701.3213	351.1643	6
13	**1230.6477**	615.8275			1212.6371	606.8222	E	**618.2842**	309.6457	601.2576	301.1324	600.2736	300.6404	5
14	**1301.6848**	651.3460			1283.6742	642.3407	A	**489.2416**	245.1244	472.2150	236.6112	471.2310	236.1191	4
15	**1430.7274**	715.8673			1412.7168	**706.8620**	E	**418.2045**	209.6059	401.1779	201.0926	400.1939	200.6006	3
16	**1544.7703**	772.8888	1527.7438	764.3755	1526.7597	763.8835	N	**289.1619**	145.0846	272.1353	136.5713			2
17							R	175.1190	88.0631	158.0924	79.5498			1

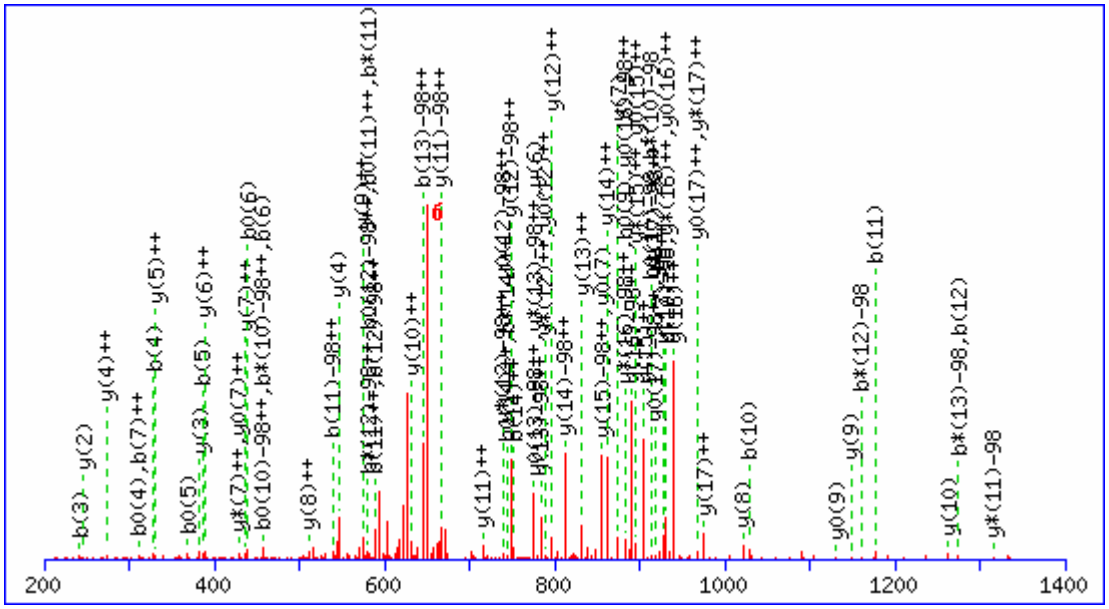
MS/MS Fragmentation of **GFVPFVPGpSPIER**



Monoisotopic mass of neutral peptide Mr(calc): 1480.7115
Variable modifications:
S9 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 60 **Expect:** 0.00017
Matches (Bold Red): 32/166 fragment ions using 30 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180			G							13
2	205.0971	103.0522			F	1424.6973	712.8523	1407.6708	704.3390	1406.6868	703.8470	12
3	**304.1656**	152.5864			V	1277.6289	**639.3181**	1260.6024	630.8048	1259.6184	630.3128	11
4	**401.2183**	201.1128			P	**1178.5605**	**589.7839**	1161.5340	581.2706	1160.5499	580.7786	10
5	**548.2867**	274.6470			F	**1081.5078**	**541.2575**	1064.4812	532.7442	1063.4972	532.2522	9
6	**647.3551**	324.1812			V	**934.4393**	**467.7233**	917.4128	459.2100	916.4288	458.7180	8
7	744.4079	372.7076			P	**835.3709**	418.1891	818.3444	409.6758	**817.3604**	409.1838	7
8	**801.4294**	**401.2183**			G	**738.3182**	369.6627	721.2916	361.1495	**720.3076**	360.6574	6
9	**968.4277**	484.7175	950.4171	475.7122	S	681.2967	341.1520	664.2702	332.6387	663.2861	332.1467	5
10	1065.4805	533.2439	1047.4699	524.2386	P	**514.2984**	257.6528	497.2718	249.1395	496.2878	248.6475	4
11	**1178.5645**	**589.7859**	1160.5540	580.7806	I	417.2456	209.1264	400.2191	200.6132	399.2350	200.1212	3
12	**1307.6071**	654.3072	1289.5966	645.3019	E	**304.1615**	152.5844	287.1350	144.0711	286.1510	143.5791	2
13					R	175.1190	88.0631	158.0924	79.5498			1

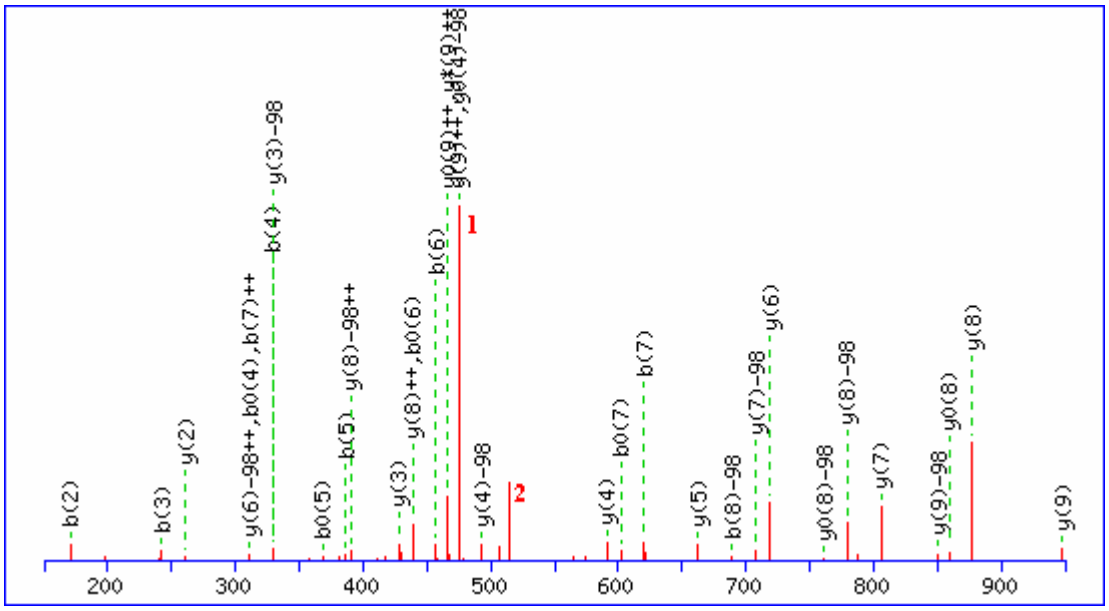
MS/MS Fragmentation of **VAASGAYp*SNKFVNDYHAR**



Monoisotopic mass of neutral peptide Mr(calc): 2048.9105
Variable modifications:
S8 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 74 **Expect:** 3.4e-06
Matches (Bold Red): 82/274 fragment ions using 110 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	100.0757	50.5415					V							18
2	171.1128	86.0600					A	1950.8494	**975.9283**	1933.8229	**967.4151**	1932.8388	**966.9231**	17
3	**242.1499**	121.5786					A	1879.8123	**940.4098**	1862.7857	**931.8965**	1861.8017	**931.4045**	16
4	**329.1819**	165.0946			**311.1714**	156.0893	S	1808.7752	**904.8912**	1791.7486	**896.3780**	1790.7646	**895.8859**	15
5	**386.2034**	193.6053			**368.1928**	184.6001	G	1721.7432	**861.3752**	1704.7166	852.8619	1703.7326	852.3699	14
6	**457.2405**	229.1239			**439.2300**	220.1186	A	1664.7217	**832.8645**	1647.6951	824.3512	1646.7111	823.8592	13
7	620.3039	**310.6556**			602.2933	301.6503	Y	1593.6846	**797.3459**	1576.6580	**788.8327**	1575.6740	**788.3406**	12
8	787.3022	394.1547			769.2916	385.1495	S	**1430.6212**	**715.8143**	1413.5947	707.3010	1412.6107	706.8090	11
9	901.3451	451.1762	884.3186	442.6629	**883.3346**	442.1709	N	**1263.6229**	**632.3151**	1246.5963	623.8018	1245.6123	623.3098	10
10	**1029.4401**	515.2237	1012.4135	506.7104	1011.4295	506.2184	K	**1149.5800**	**575.2936**	1132.5534	566.7803	**1131.5694**	566.2883	9
11	**1176.5085**	**588.7579**	1159.4820	**580.2446**	1158.4979	**579.7526**	F	**1021.4850**	**511.2461**	1004.4585	502.7329	1003.4744	502.2409	8
12	**1275.5769**	638.2921	1258.5504	629.7788	1257.5664	629.2868	V	**874.4166**	**437.7119**	857.3900	**429.1987**	**856.4060**	**428.7067**	7
13	1389.6199	695.3136	1372.5933	686.8003	1371.6093	686.3083	N	**775.3482**	**388.1777**	758.3216	379.6645	757.3376	379.1724	6
14	**1504.6468**	**752.8270**	1487.6202	**744.3138**	1486.6362	**743.8218**	D	661.3053	**331.1563**	644.2787	322.6430	643.2947	322.1510	5
15	1667.7101	834.3587	1650.6836	825.8454	1649.6996	825.3534	Y	**546.2783**	**273.6428**	529.2518	265.1295			4
16	1804.7690	902.8882	1787.7425	894.3749	1786.7585	893.8829	H	**383.2150**	192.1111	366.1884	183.5979			3
17	1875.8061	938.4067	1858.7796	929.8934	1857.7956	929.4014	A	**246.1561**	123.5817	229.1295	115.0684			2
18							R	175.1190	88.0631	158.0924	79.5498			1

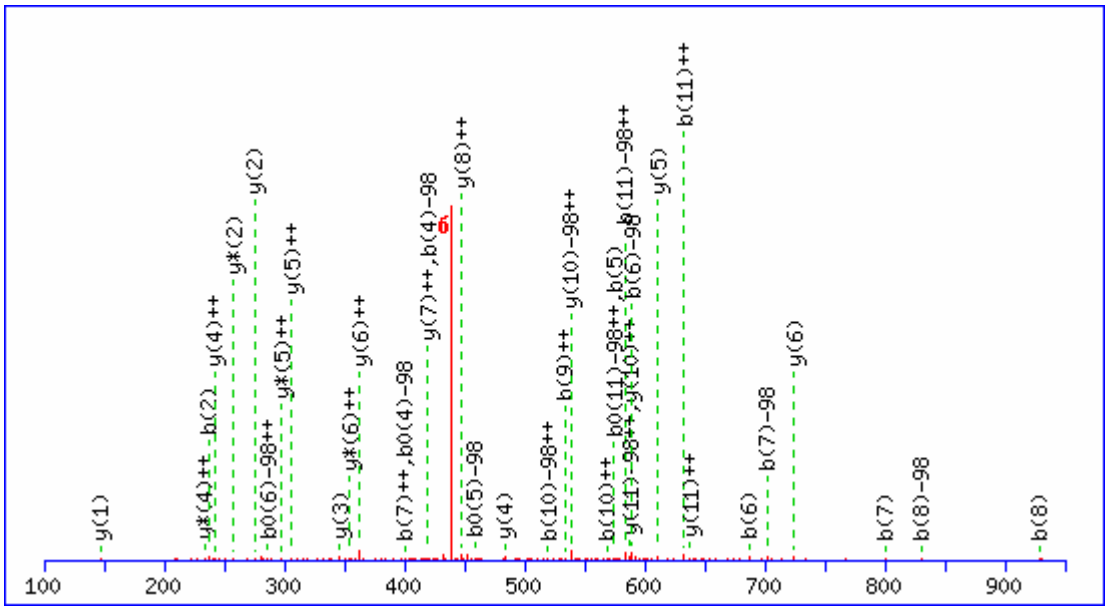
MS/MS Fragmentation of **VAASGAYp*SNK**



Monoisotopic mass of neutral peptide Mr(calc): 1046.4434
Variable modifications:
S8 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 85 **Expect:** 1.2e-07
Matches (Bold Red): 34/134 fragment ions using 32 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	100.0757	50.5415					V							10
2	**171.1128**	86.0600					A	**948.3822**	**474.6948**	931.3557	**466.1815**	930.3717	**465.6895**	9
3	**242.1499**	121.5786					A	**877.3451**	**439.1762**	860.3186	430.6629	**859.3346**	430.1709	8
4	**329.1819**	165.0946			**311.1714**	156.0893	S	**806.3080**	403.6576	789.2815	395.1444	788.2975	394.6524	7
5	**386.2034**	193.6053			**368.1928**	184.6001	G	**719.2760**	360.1416	702.2494	351.6284	701.2654	351.1364	6
6	**457.2405**	229.1239			**439.2300**	220.1186	A	**662.2545**	331.6309	645.2280	323.1176	644.2440	322.6256	5
7	**620.3039**	**310.6556**			**602.2933**	301.6503	Y	**591.2174**	296.1123	574.1909	287.5991	573.2068	287.1071	4
8	787.3022	394.1547			769.2916	385.1495	S	**428.1541**	214.5807	411.1275	206.0674	410.1435	205.5754	3
9	901.3451	451.1762	884.3186	442.6629	883.3346	442.1709	N	**261.1557**	131.0815	244.1292	122.5682			2
10							K	147.1128	74.0600	130.0863	65.5468			1

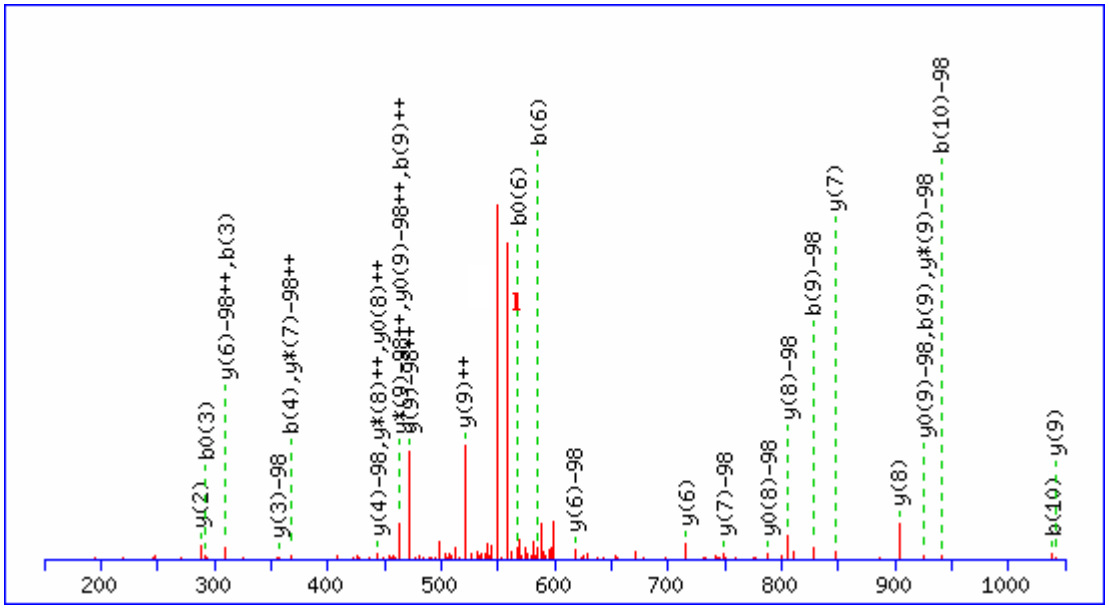
MS/MS Fragmentation of **HVISGILQHAQK**



Monoisotopic mass of neutral peptide Mr(calc): 1409.7180
Variable modifications:
S4 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 37 **Expect:** 0.0077
Matches (Bold Red): 38/154 fragment ions using 60 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	138.0662	69.5367					H							12
2	**237.1346**	119.0709					V	1273.6664	**637.3368**	1256.6399	628.8236	1255.6558	628.3316	11
3	350.2187	175.6130					I	1174.5980	**587.8026**	1157.5714	579.2894	1156.5874	578.7974	10
4	517.2170	259.1121			499.2065	250.1069	S	1061.5139	531.2606	1044.4874	522.7473	1043.5034	522.2553	9
5	**574.2385**	287.6229			556.2279	278.6176	G	894.5156	**447.7614**	877.4890	439.2482			8
6	**687.3225**	344.1649			669.3120	335.1596	I	837.4941	**419.2507**	820.4676	410.7374			7
7	**800.4066**	**400.7069**			782.3960	391.7017	L	**724.4101**	**362.7087**	707.3835	**354.1954**			6
8	**928.4652**	464.7362	911.4386	456.2230	910.4546	455.7309	Q	**611.3260**	**306.1666**	594.2994	**297.6534**			5
9	1065.5241	**533.2657**	1048.4976	524.7524	1047.5135	524.2604	H	**483.2674**	**242.1373**	466.2409	**233.6241**			4
10	1136.5612	**568.7842**	1119.5347	560.2710	1118.5506	559.7790	A	**346.2085**	173.6079	329.1819	165.0946			3
11	1264.6198	**632.8135**	1247.5932	624.3003	1246.6092	623.8083	Q	**275.1714**	138.0893	**258.1448**	129.5761			2
12							K	**147.1128**	74.0600	130.0863	65.5468			1

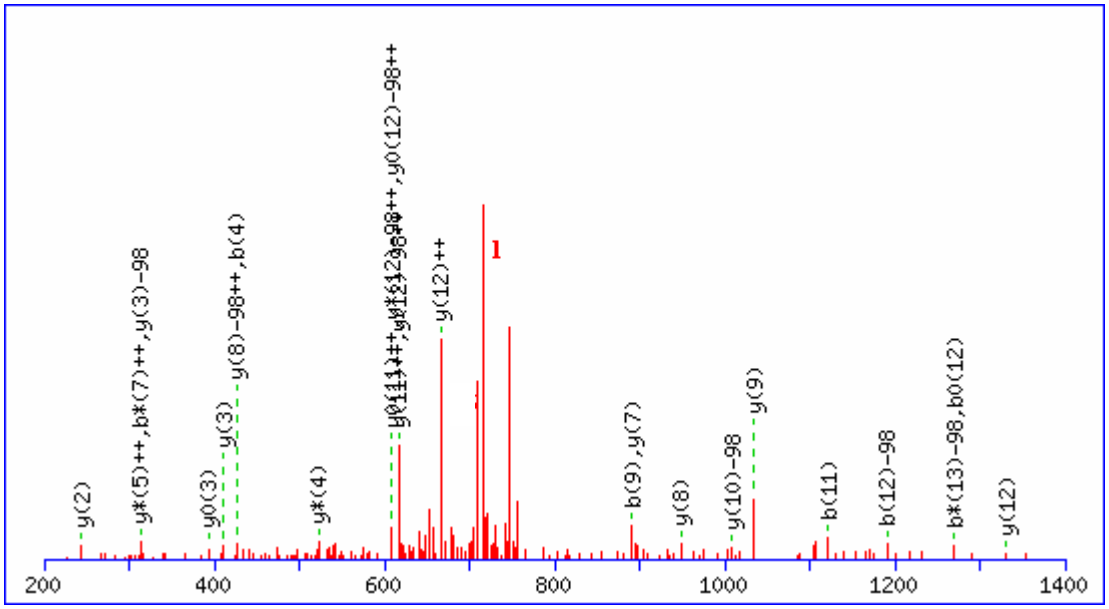
MS/MS Fragmentation of **TAHGMSSSp*SLR**



Monoisotopic mass of neutral peptide Mr(calc): 1212.4958
Variable modifications:
S9 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 59 **Expect:** 4.3e-05
Matches (Bold Red): 31/152 fragment ions using 32 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	102.0550	51.5311	84.0444	42.5258	T							11
2	173.0921	87.0497	155.0815	78.0444	A	1014.4785	507.7429	997.4520	499.2296	996.4680	498.7376	10
3	**310.1510**	155.5791	**292.1404**	146.5738	H	943.4414	**472.2243**	**926.4149**	**463.7111**	**925.4308**	**463.2191**	9
4	**367.1724**	184.0899	349.1619	175.0846	G	**806.3825**	403.6949	789.3560	395.1816	**788.3719**	394.6896	8
5	498.2129	249.6101	480.2024	240.6048	M	**749.3610**	375.1842	732.3345	**366.6709**	731.3505	366.1789	7
6	**585.2449**	293.1261	**567.2344**	284.1208	S	**618.3206**	**309.6639**	601.2940	301.1506	600.3100	300.6586	6
7	672.2770	336.6421	654.2664	327.6368	S	531.2885	266.1479	514.2620	257.6346	513.2780	257.1426	5
8	759.3090	380.1581	741.2984	371.1529	S	**444.2565**	222.6319	427.2300	214.1186	426.2459	213.6266	4
9	**828.3305**	414.6689	810.3199	405.6636	S	**357.2245**	179.1159	340.1979	170.6026	339.2139	170.1106	3
10	**941.4145**	471.2109	923.4040	462.2056	L	**288.2030**	144.6051	271.1765	136.0919			2
11					R	175.1190	88.0631	158.0924	79.5498			1

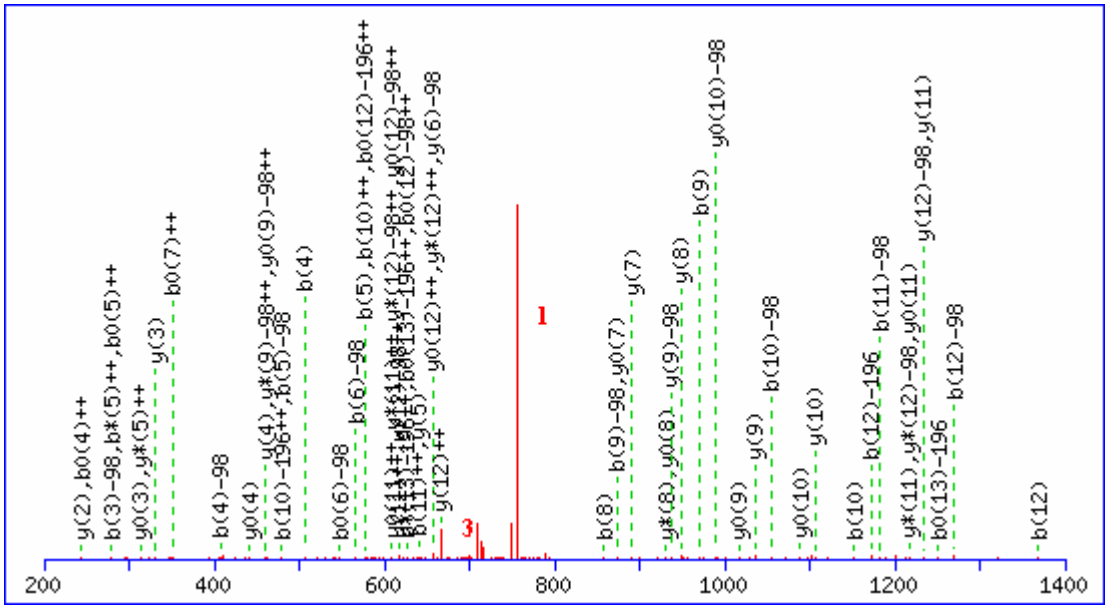
MS/MS Fragmentation of **LSPQASGHLTEpSPK**



Monoisotopic mass of neutral peptide Mr(calc): 1530.7079
Variable modifications:
S12 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 45 **Expect:** 0.0019
Matches (Bold Red): 25/222 fragment ions using 25 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							14
2	201.1234	101.0653			183.1128	92.0600	S	1418.6311	709.8192	1401.6046	701.3059	1400.6206	700.8139	13
3	298.1761	149.5917			280.1656	140.5864	P	**1331.5991**	**666.3032**	1314.5726	657.7899	1313.5886	657.2979	12
4	**426.2347**	213.6210	409.2082	205.1077	408.2241	204.6157	Q	1234.5464	**617.7768**	1217.5198	609.2635	1216.5358	**608.7715**	11
5	497.2718	249.1395	480.2453	240.6263	479.2613	240.1343	A	1106.4878	553.7475	1089.4612	545.2343	1088.4772	544.7422	10
6	584.3039	292.6556	567.2773	284.1423	566.2933	283.6503	S	**1035.4507**	518.2290	1018.4241	509.7157	1017.4401	509.2237	9
7	641.3253	321.1663	624.2988	**312.6530**	623.3148	312.1610	G	**948.4186**	474.7130	931.3921	466.1997	930.4081	465.7077	8
8	778.3842	389.6958	761.3577	381.1825	760.3737	380.6905	H	**891.3972**	446.2022	874.3706	437.6889	873.3866	437.1969	7
9	**891.4683**	446.2378	874.4417	437.7245	873.4577	437.2325	L	754.3383	377.6728	737.3117	369.1595	736.3277	368.6675	6
10	992.5160	496.7616	975.4894	488.2483	974.5054	487.7563	T	641.2542	321.1307	624.2276	**312.6175**	623.2436	312.1255	5
11	**1121.5586**	561.2829	1104.5320	552.7696	1103.5480	552.2776	E	540.2065	270.6069	**523.1800**	262.0936	522.1959	261.6016	4
12	1288.5569	644.7821	1271.5304	636.2688	**1270.5464**	635.7768	S	**411.1639**	206.0856	394.1374	197.5723	**393.1534**	197.0803	3
13	1385.6097	693.3085	1368.5831	684.7952	1367.5991	684.3032	P	**244.1656**	122.5864	227.1390	114.0731			2
14							K	147.1128	74.0600	130.0863	65.5468			1

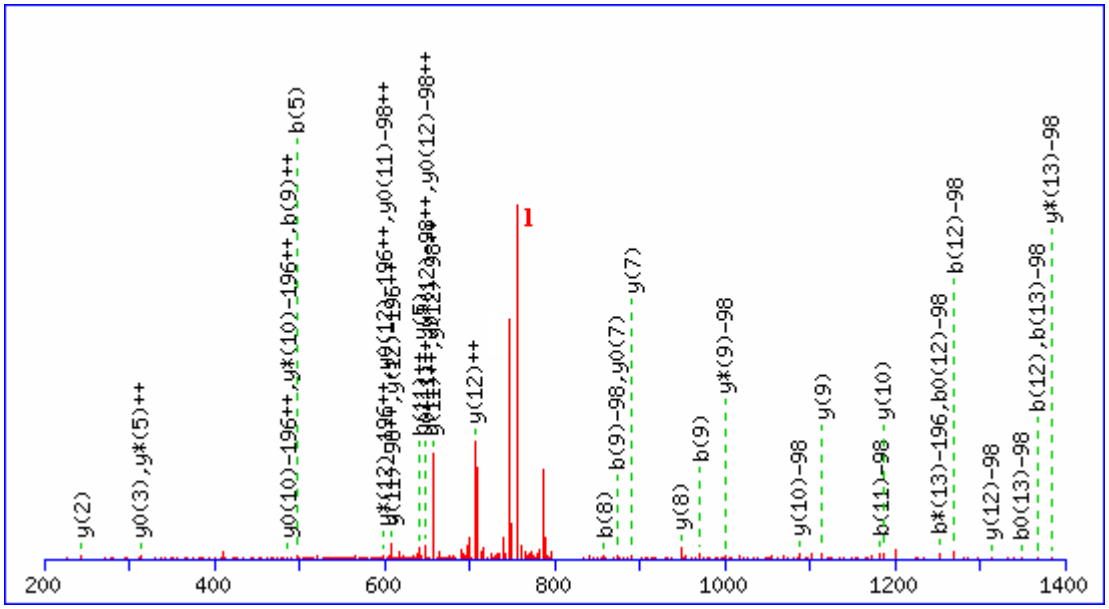
MS/MS Fragmentation of **LpSPQASGHLp*TESPK**



Monoisotopic mass of neutral peptide Mr(calc): 1610.6742
Variable modifications:
S2 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
T10 : Phospho (T), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 51 **Expect:** 0.00043
Matches (Bold Red): 67/326 fragment ions using 66 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							14
2	183.1128	92.0600			165.1022	83.0548	S	1400.6206	700.8139	1383.5940	692.3006	1382.6100	691.8086	13
3	**280.1656**	140.5864			262.1550	131.5811	P	1331.5991	**666.3032**	1314.5725	**657.7899**	1313.5885	**657.2979**	12
4	**408.2241**	204.6157	391.1976	196.1024	390.2136	195.6104	Q	**1234.5463**	**617.7768**	**1217.5198**	**609.2635**	**1216.5358**	**608.7715**	11
5	**479.2612**	240.1343	462.2347	231.6210	461.2507	231.1290	A	**1106.4878**	553.7475	1089.4612	545.2342	**1088.4772**	544.7422	10
6	**566.2933**	283.6503	549.2667	275.1370	**548.2827**	274.6450	S	**1035.4506**	518.2290	1018.4241	509.7157	**1017.4401**	509.2237	9
7	623.3147	312.1610	606.2882	303.6477	605.3042	303.1557	G	**948.4186**	474.7129	**931.3921**	466.1997	**930.4081**	465.7077	8
8	760.3736	380.6905	743.3471	372.1772	742.3631	371.6852	H	**891.3972**	446.2022	874.3706	437.6889	**873.3866**	437.1969	7
9	**873.4577**	437.2325	856.4312	428.7192	855.4471	428.2272	L	754.3382	377.6728	737.3117	369.1595	736.3277	368.6675	6
10	**1054.4717**	527.7395	1037.4452	519.2262	1036.4611	518.7342	T	**641.2542**	321.1307	624.2276	**312.6175**	623.2436	312.1254	5
11	**1183.5143**	592.2608	1166.4877	583.7475	1165.5037	583.2555	E	**460.2402**	230.6237	443.2136	222.1105	**442.2296**	221.6184	4
12	**1270.5463**	635.7768	1253.5198	627.2635	1252.5358	**626.7715**	S	**331.1976**	166.1024	314.1710	157.5892	**313.1870**	157.0972	3
13	1367.5991	684.3032	1350.5725	675.7899	1349.5885	675.2979	P	**244.1656**	122.5864	227.1390	114.0731			2
14							K	147.1128	74.0600	130.0863	65.5468			1

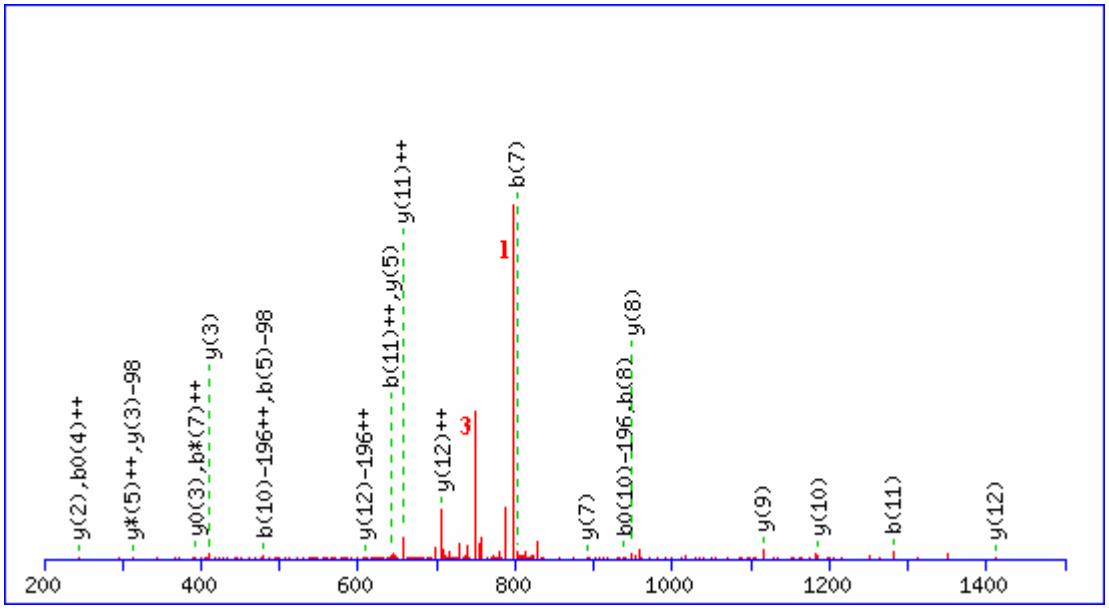
MS/MS Fragmentation of **LSPQAp*SGHLp*TESPK**



Monoisotopic mass of neutral peptide Mr(calc): 1610.6742
Variable modifications:
S6 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
T10 : Phospho (T), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 47 **Expect:** 0.00078
Matches (Bold Red): 53/354 fragment ions using 41 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							14
2	201.1234	101.0653			183.1128	92.0600	S	1400.6206	700.8139	**1383.5940**	692.3006	1382.6100	691.8086	13
3	298.1761	149.5917			280.1656	140.5864	P	**1313.5885**	**657.2979**	1296.5620	**648.7846**	1295.5780	**648.2926**	12
4	426.2347	213.6210	409.2082	205.1077	408.2241	204.6157	Q	1216.5358	**608.7715**	1199.5092	600.2582	1198.5252	**599.7662**	11
5	**497.2718**	249.1395	480.2453	240.6263	479.2613	240.1343	A	**1088.4772**	544.7422	1071.4506	536.2290	1070.4666	535.7369	10
6	566.2933	283.6503	549.2667	275.1370	548.2827	274.6450	S	1017.4401	509.2237	**1000.4135**	500.7104	999.4295	500.2184	9
7	623.3147	312.1610	606.2882	303.6477	605.3042	303.1557	G	**948.4186**	474.7129	931.3921	466.1997	930.4081	465.7077	8
8	760.3736	380.6905	743.3471	372.1772	742.3631	371.6852	H	**891.3972**	446.2022	874.3706	437.6889	**873.3866**	437.1969	7
9	**873.4577**	437.2325	856.4312	428.7192	855.4471	428.2272	L	754.3382	377.6728	737.3117	369.1595	736.3277	368.6675	6
10	1054.4717	527.7395	1037.4452	519.2262	1036.4611	518.7342	T	**641.2542**	321.1307	624.2276	**312.6175**	623.2436	312.1254	5
11	**1183.5143**	592.2608	1166.4877	583.7475	1165.5037	583.2555	E	460.2402	230.6237	443.2136	222.1105	442.2296	221.6184	4
12	**1270.5463**	635.7768	1253.5198	627.2635	**1252.5358**	626.7715	S	331.1976	166.1024	314.1710	157.5892	**313.1870**	157.0972	3
13	**1367.5991**	684.3032	1350.5725	675.7899	**1349.5885**	675.2979	P	**244.1656**	122.5864	227.1390	114.0731			2
14							K	147.1128	74.0600	130.0863	65.5468			1

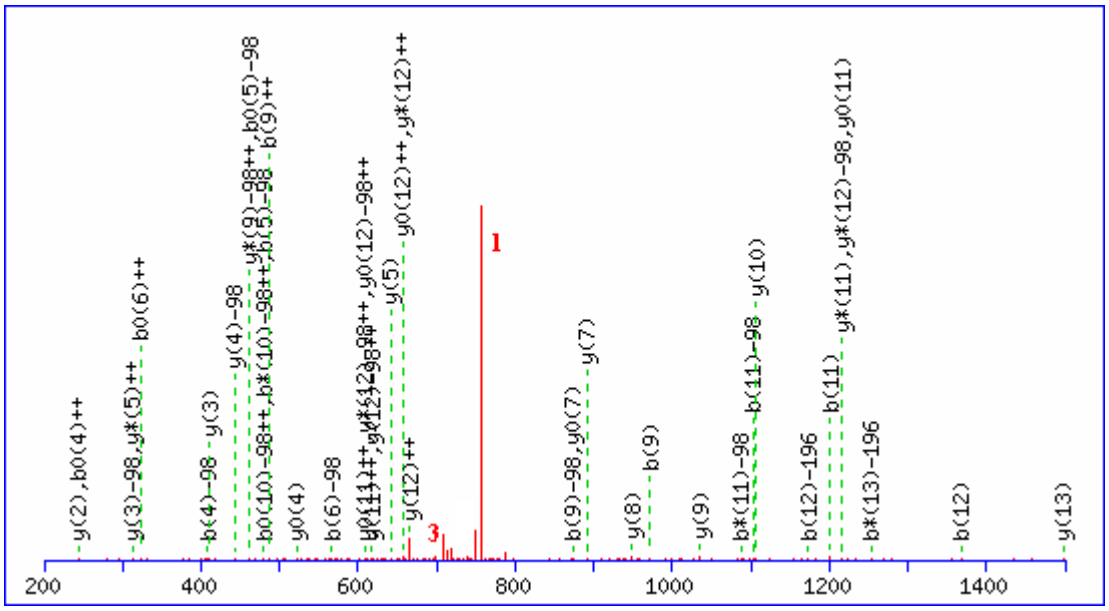
MS/MS Fragmentation of **Lp*SPQAp*SGHLTEpSPK**



Monoisotopic mass of neutral peptide Mr(calc): 1690.6406
Variable modifications:
S2 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
S6 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
S12 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 48 **Expect:** 0.00043
Matches (Bold Red): 23/278 fragment ions using 38 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							14
2	281.0897	141.0485			263.0791	132.0432	S	1578.5638	789.7855	1561.5372	781.2723	1560.5532	780.7803	13
3	378.1425	189.5749			360.1319	180.5696	P	**1411.5654**	**706.2864**	1394.5389	697.7731	1393.5549	697.2811	12
4	506.2010	253.6042	489.1745	245.0909	488.1905	**244.5989**	Q	1314.5127	**657.7600**	1297.4861	649.2467	1296.5021	648.7547	11
5	577.2381	289.1227	560.2116	280.6094	559.2276	280.1174	A	**1186.4541**	593.7307	1169.4275	585.2174	1168.4435	584.7254	10
6	744.2365	372.6219	727.2100	364.1086	726.2259	363.6166	S	**1115.4170**	558.2121	1098.3904	549.6989	1097.4064	549.2068	9
7	**801.2580**	401.1326	784.2314	**392.6193**	783.2474	392.1273	G	**948.4186**	474.7130	931.3921	466.1997	930.4081	465.7077	8
8	**938.3169**	469.6621	921.2903	461.1488	920.3063	460.6568	H	**891.3972**	446.2022	874.3706	437.6889	873.3866	437.1969	7
9	1051.4009	526.2041	1034.3744	517.6908	1033.3904	517.1988	L	754.3383	377.6728	737.3117	369.1595	736.3277	368.6675	6
10	1152.4486	576.7279	1135.4221	568.2147	1134.4381	567.7227	T	**641.2542**	321.1307	624.2276	**312.6175**	623.2436	312.1255	5
11	**1281.4912**	**641.2492**	1264.4647	632.7360	1263.4806	632.2440	E	540.2065	270.6069	523.1800	262.0936	522.1959	261.6016	4
12	1448.4896	724.7484	1431.4630	716.2351	1430.4790	715.7431	S	**411.1639**	206.0856	394.1374	197.5723	**393.1534**	197.0803	3
13	1545.5423	773.2748	1528.5158	764.7615	1527.5318	764.2695	P	**244.1656**	122.5864	227.1390	114.0731			2
14							K	147.1128	74.0600	130.0863	65.5468			1

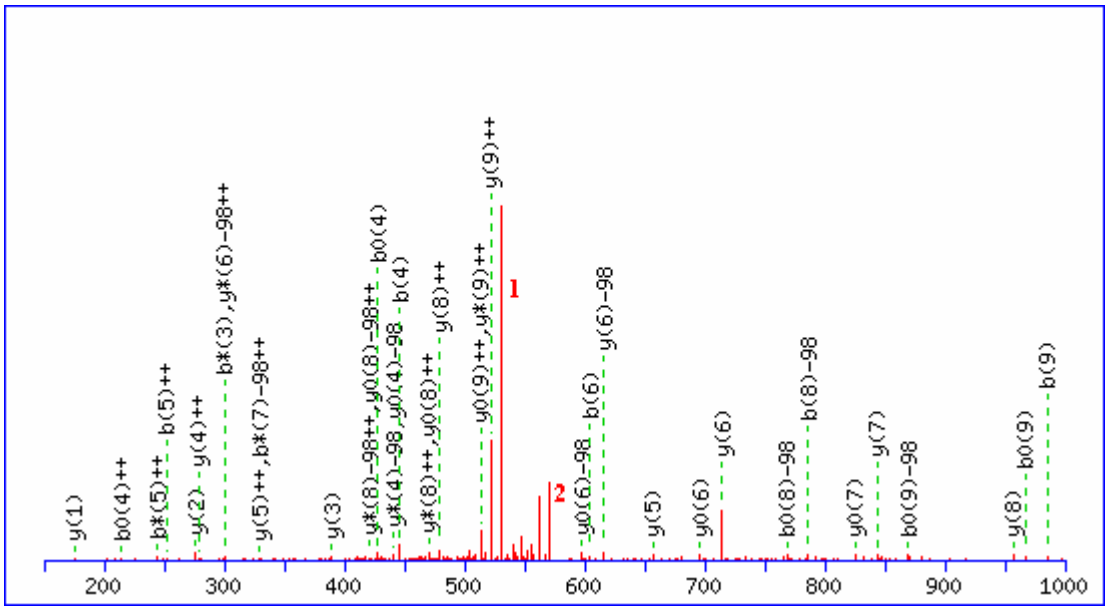
MS/MS Fragmentation of **Lp*SPQASGHLTEp*SPK**



Monoisotopic mass of neutral peptide Mr(calc): 1610.6742
Variable modifications:
S2 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769
S12 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 28 **Expect:** 0.043
Matches (Bold Red): 42/278 fragment ions using 51 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							14
2	281.0897	141.0485			263.0791	132.0432	S	**1498.5975**	749.8024	1481.5709	741.2891	1480.5869	740.7971	13
3	378.1425	189.5749			360.1319	180.5696	P	1331.5991	**666.3032**	1314.5726	**657.7899**	1313.5886	**657.2979**	12
4	506.2010	253.6042	489.1745	245.0909	488.1905	**244.5989**	Q	1234.5464	**617.7768**	**1217.5198**	609.2635	**1216.5358**	**608.7715**	11
5	577.2381	289.1227	560.2116	280.6094	559.2276	280.1174	A	**1106.4878**	553.7475	1089.4612	545.2343	1088.4772	544.7422	10
6	664.2702	332.6387	647.2436	324.1255	646.2596	**323.6334**	S	**1035.4507**	518.2290	1018.4241	509.7157	1017.4401	509.2237	9
7	721.2916	361.1495	704.2651	352.6362	703.2811	352.1442	G	**948.4186**	474.7130	931.3921	466.1997	930.4081	465.7077	8
8	858.3506	429.6789	841.3240	421.1656	840.3400	420.6736	H	**891.3972**	446.2022	874.3706	437.6889	**873.3866**	437.1969	7
9	**971.4346**	**486.2209**	954.4081	477.7077	953.4241	477.2157	L	754.3383	377.6728	737.3117	369.1595	736.3277	368.6675	6
10	1072.4823	536.7448	1055.4557	528.2315	1054.4717	527.7395	T	**641.2542**	321.1307	624.2276	**312.6175**	623.2436	312.1255	5
11	**1201.5249**	601.2661	1184.4983	592.7528	1183.5143	592.2608	E	540.2065	270.6069	523.1800	262.0936	**522.1959**	261.6016	4
12	**1368.5232**	684.7653	1351.4967	676.2520	1350.5127	675.7600	S	**411.1639**	206.0856	394.1374	197.5723	393.1534	197.0803	3
13	1465.5760	733.2916	1448.5495	724.7784	1447.5654	724.2864	P	**244.1656**	122.5864	227.1390	114.0731			2
14							K	147.1128	74.0600	130.0863	65.5468			1

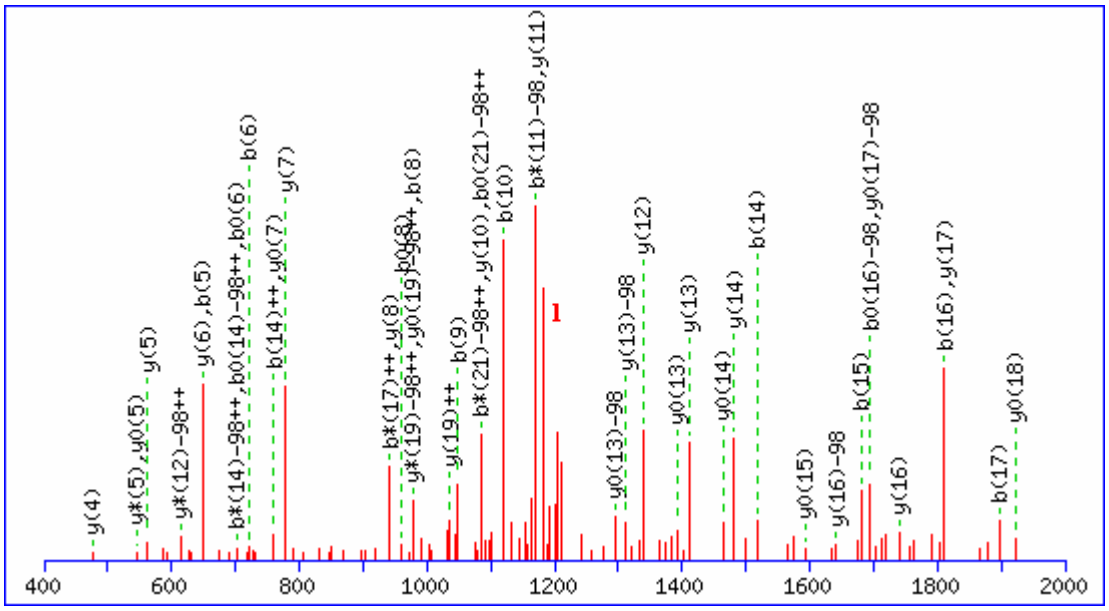
MS/MS Fragmentation of **DSNEGTpSLTR**



Monoisotopic mass of neutral peptide Mr(calc): 1158.4554
Variable modifications:
S7 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 39 **Expect:** 0.0042
Matches (Bold Red): 37/156 fragment ions using 46 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	116.0342	58.5207			98.0237	49.5155	D							10
2	203.0662	102.0368			185.0557	93.0315	S	1044.4357	**522.7215**	1027.4092	**514.2082**	1026.4252	**513.7162**	9
3	317.1092	159.0582	**300.0826**	150.5450	299.0986	150.0529	N	**957.4037**	**479.2055**	940.3772	**470.6922**	939.3931	**470.2002**	8
4	**446.1518**	223.5795	429.1252	215.0662	**428.1412**	**214.5742**	E	**843.3608**	422.1840	826.3342	413.6708	**825.3502**	413.1787	7
5	503.1732	**252.0903**	486.1467	**243.5770**	485.1627	243.0850	G	**714.3182**	357.6627	697.2916	349.1495	**696.3076**	348.6575	6
6	**604.2209**	302.6141	587.1944	294.1008	586.2103	293.6088	T	**657.2967**	**329.1520**	640.2702	320.6387	639.2862	320.1467	5
7	771.2193	386.1133	754.1927	377.6000	753.2087	377.1080	S	556.2490	**278.6282**	539.2225	270.1149	538.2385	269.6229	4
8	884.3033	442.6553	867.2768	434.1420	866.2928	433.6500	L	**389.2507**	195.1290	372.2241	186.6157	371.2401	186.1237	3
9	**985.3510**	493.1791	968.3245	484.6659	**967.3404**	484.1739	T	**276.1666**	138.5870	259.1401	130.0737	258.1561	129.5817	2
10							R	**175.1190**	88.0631	158.0924	79.5498			1

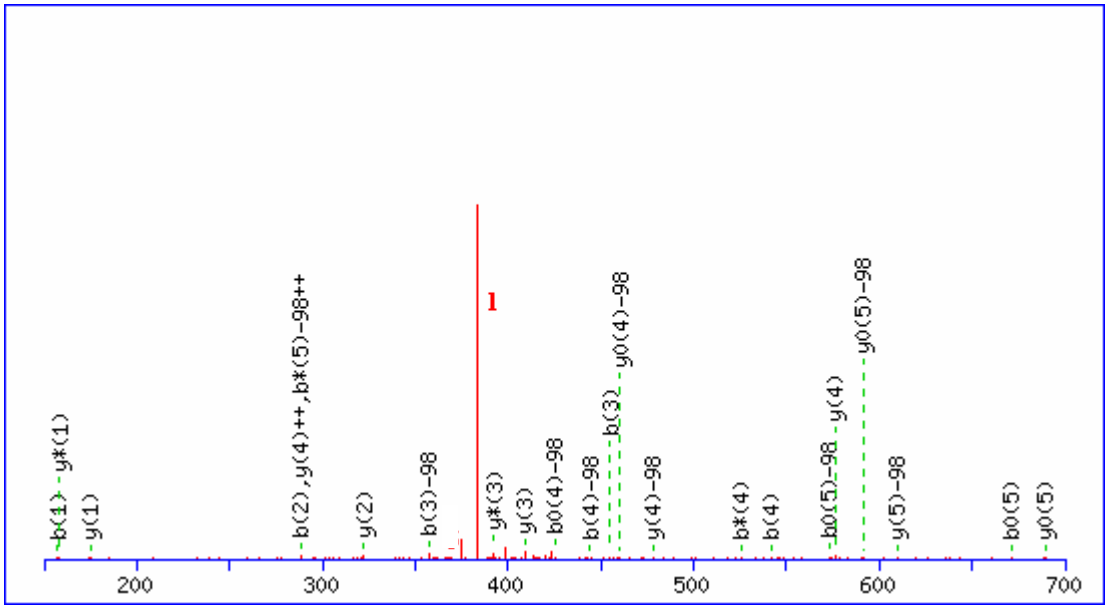
MS/MS Fragmentation of **FIQMEAEEAAp*SSGSYESSNEGR**



Monoisotopic mass of neutral peptide Mr(calc): 2457.9631
Variable modifications:
S11 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 76 **Expect:** 7.2e-07
Matches (Bold Red): 47/362 fragment ions using 46 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	148.0757	74.5415					F							22
2	261.1598	131.0835					I	2311.9020	1156.4547	2294.8755	1147.9414	2293.8915	1147.4494	21
3	389.2183	195.1128	372.1918	186.5995			Q	2198.8180	1099.9126	2181.7914	1091.3993	2180.8074	1090.9073	20
4	520.2588	260.6330	503.2323	252.1198			M	2070.7594	**1035.8833**	2053.7328	1027.3701	2052.7488	1026.8781	19
5	**649.3014**	325.1543	632.2749	316.6411	631.2908	316.1491	E	1939.7189	970.3631	1922.6924	961.8498	**1921.7083**	961.3578	18
6	**720.3385**	360.6729	703.3120	352.1596	**702.3280**	351.6676	A	**1810.6763**	905.8418	1793.6498	897.3285	1792.6657	896.8365	17
7	849.3811	425.1942	832.3546	416.6809	831.3706	416.1889	E	**1739.6392**	870.3232	1722.6127	861.8100	1721.6286	861.3180	16
8	**978.4237**	489.7155	961.3972	481.2022	**960.4131**	480.7102	E	1610.5966	805.8019	1593.5701	797.2887	**1592.5860**	796.7967	15
9	**1049.4608**	525.2341	1032.4343	516.7208	1031.4503	516.2288	A	**1481.5540**	741.2806	1464.5275	732.7674	**1463.5434**	732.2754	14
10	**1120.4979**	560.7526	1103.4714	552.2393	1102.4874	551.7473	A	**1410.5169**	705.7621	1393.4904	697.2488	**1392.5063**	696.7568	13
11	1287.4963	644.2518	1270.4697	635.7385	1269.4857	635.2465	S	**1339.4798**	670.2435	1322.4532	661.7303	1321.4692	661.2382	12
12	1374.5283	687.7678	1357.5018	679.2545	1356.5178	678.7625	S	**1172.4814**	586.7444	1155.4549	578.2311	1154.4709	577.7391	11
13	1431.5498	716.2785	1414.5232	707.7653	1413.5392	707.2732	G	**1085.4494**	543.2283	1068.4229	534.7151	1067.4388	534.2231	10
14	**1518.5818**	**759.7945**	1501.5553	751.2813	1500.5712	750.7893	S	1028.4279	514.7176	1011.4014	506.2043	1010.4174	505.7123	9
15	**1681.6451**	841.3262	1664.6186	832.8129	1663.6346	832.3209	Y	**941.3959**	471.2016	924.3694	462.6883	923.3853	462.1963	8
16	**1810.6877**	905.8475	1793.6612	897.3342	1792.6772	896.8422	E	**778.3326**	389.6699	761.3060	381.1567	**760.3220**	380.6646	7
17	**1897.7198**	949.3635	1880.6932	**940.8502**	1879.7092	940.3582	S	**649.2900**	325.1486	632.2634	316.6354	631.2794	316.1434	6
18	1984.7518	992.8795	1967.7252	984.3663	1966.7412	983.8742	S	**562.2580**	281.6326	**545.2314**	273.1193	**544.2474**	272.6273	5
19	2098.7947	1049.9010	2081.7682	1041.3877	2080.7841	1040.8957	N	**475.2259**	238.1166	458.1994	229.6033	457.2154	229.1113	4
20	2227.8373	1114.4223	2210.8108	1105.9090	2209.8267	1105.4170	E	361.1830	181.0951	344.1565	172.5819	343.1724	172.0899	3
21	2284.8588	1142.9330	2267.8322	1134.4197	2266.8482	1133.9277	G	232.1404	116.5738	215.1139	108.0606			2
22							R	175.1190	88.0631	158.0924	79.5498			1

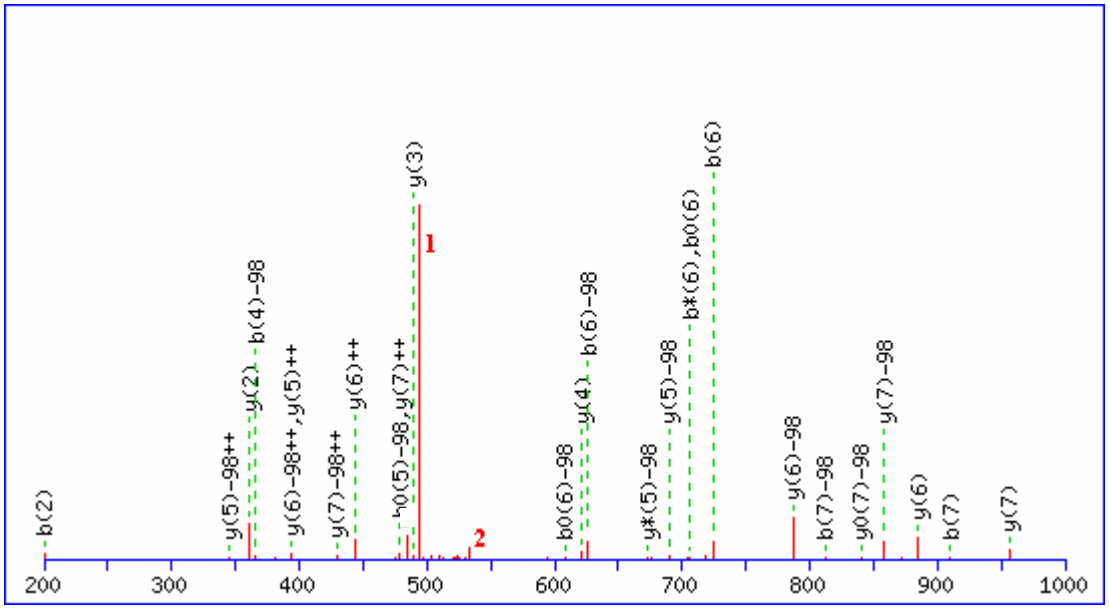
MS/MS Fragmentation of **RMpSSFR**



Monoisotopic mass of neutral peptide Mr(calc): 862.3520
Variable modifications:
S3 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 30 **Expect:** 0.011
Matches (Bold Red): 25/82 fragment ions using 49 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	**157.1084**	79.0578	140.0818	70.5446			R							6
2	**288.1489**	144.5781	271.1223	136.0648			M	**609.2813**	305.1443	592.2548	296.6310	**591.2708**	296.1390	5
3	**357.1703**	179.0888	340.1438	170.5755	339.1598	170.0835	S	**478.2408**	239.6241	461.2143	231.1108	**460.2303**	230.6188	4
4	**444.2024**	222.6048	427.1758	214.0915	**426.1918**	213.5995	S	**409.2194**	205.1133	**392.1928**	196.6001	391.2088	196.1081	3
5	**591.2708**	296.1390	574.2442	**287.6257**	**573.2602**	287.1337	F	**322.1874**	161.5973	305.1608	153.0840			2
6							R	**175.1190**	88.0631	**158.0924**	79.5498			1

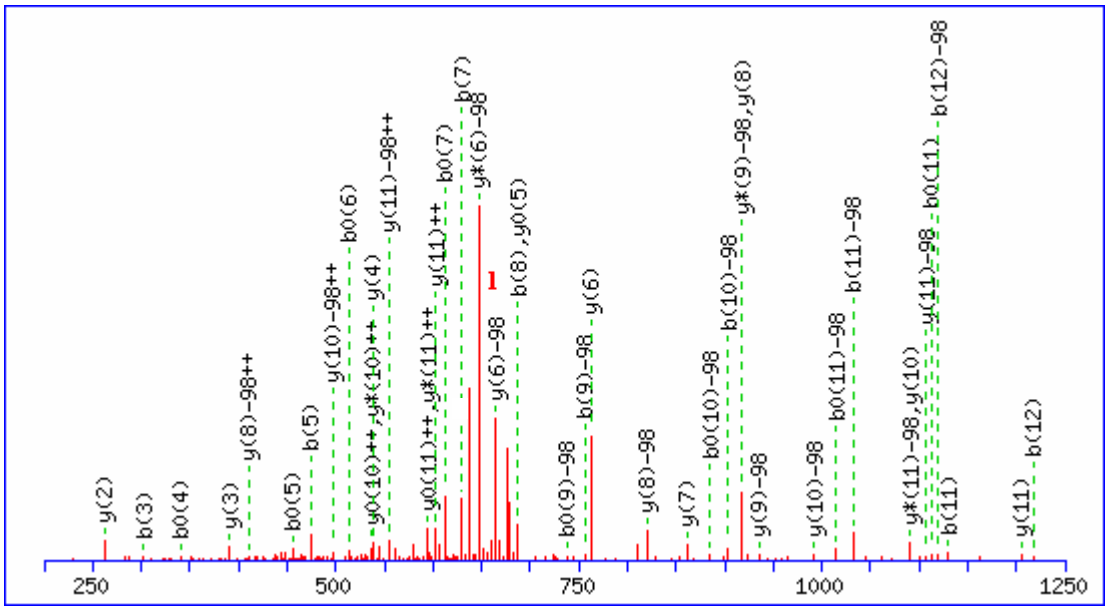
MS/MS Fragmentation of **KAPpSMEWR**



Monoisotopic mass of neutral peptide Mr(calc): 1083.4572
Variable modifications:
S4 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 43 **Expect:** 0.0017
Matches (Bold Red): 26/116 fragment ions using 36 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	129.1022	65.0548	112.0757	56.5415			K							8
2	**200.1393**	100.5733	183.1128	92.0600			A	**858.3927**	**429.7000**	841.3661	421.1867	**840.3821**	420.6947	7
3	297.1921	149.0997	280.1656	140.5864			P	**787.3555**	**394.1814**	770.3290	385.6681	769.3450	385.1761	6
4	**366.2136**	183.6104	349.1870	175.0971	348.2030	174.6051	S	**690.3028**	**345.6550**	**673.2762**	337.1418	672.2922	336.6498	5
5	497.2540	249.1307	480.2275	240.6174	**479.2435**	240.1254	M	**621.2813**	311.1443	604.2548	302.6310	603.2708	302.1390	4
6	**626.2966**	313.6520	609.2701	305.1387	**608.2861**	304.6467	E	**490.2409**	245.6241	473.2143	237.1108	472.2303	236.6188	3
7	**812.3759**	406.6916	795.3494	398.1783	794.3654	397.6863	W	**361.1983**	181.1028	344.1717	172.5895			2
8							R	175.1190	88.0631	158.0924	79.5498			1

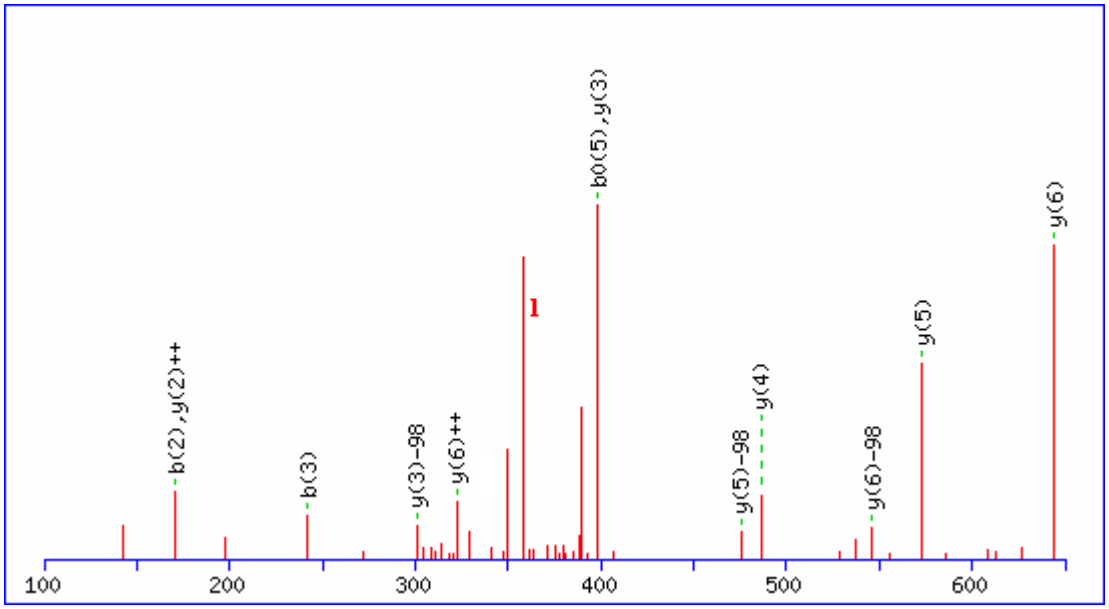
MS/MS Fragmentation of **GEDGDGVGpSFESR**



Monoisotopic mass of neutral peptide Mr(calc): 1390.5038
Variable modifications:
S9 : Phospho (ST), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 55 **Expect:** 5.8e-05
Matches (Bold Red): 43/180 fragment ions using 55 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180			G							13
2	187.0713	94.0393	169.0608	85.0340	E	1236.5127	618.7600	1219.4862	610.2467	1218.5022	609.7547	12
3	**302.0983**	151.5528	284.0877	142.5475	D	**1107.4701**	**554.2387**	**1090.4436**	545.7254	1089.4596	545.2334	11
4	359.1197	180.0635	**341.1092**	171.0582	G	**992.4432**	**496.7252**	975.4166	488.2120	974.4326	487.7200	10
5	**474.1467**	237.5770	**456.1361**	228.5717	D	**935.4217**	468.2145	**918.3952**	459.7012	917.4112	459.2092	9
6	531.1681	266.0877	**513.1576**	257.0824	G	**820.3948**	**410.7010**	803.3682	402.1878	802.3842	401.6957	8
7	**630.2366**	315.6219	**612.2260**	306.6166	V	763.3733	382.1903	746.3468	373.6770	745.3628	373.1850	7
8	**687.2580**	344.1327	669.2475	335.1274	G	**664.3049**	332.6561	**647.2784**	324.1428	646.2943	323.6508	6
9	**756.2795**	378.6434	**738.2689**	369.6381	S	607.2834	304.1454	590.2569	295.6321	589.2729	295.1401	5
10	**903.3479**	452.1776	**885.3373**	443.1723	F	**538.2620**	269.6346	521.2354	261.1214	520.2514	260.6293	4
11	**1032.3905**	516.6989	**1014.3799**	507.6936	E	**391.1936**	196.1004	374.1670	187.5871	373.1830	187.0951	3
12	**1119.4225**	560.2149	1101.4120	551.2096	S	**262.1510**	131.5791	245.1244	123.0659	244.1404	122.5738	2
13					R	175.1190	88.0631	158.0924	79.5498			1

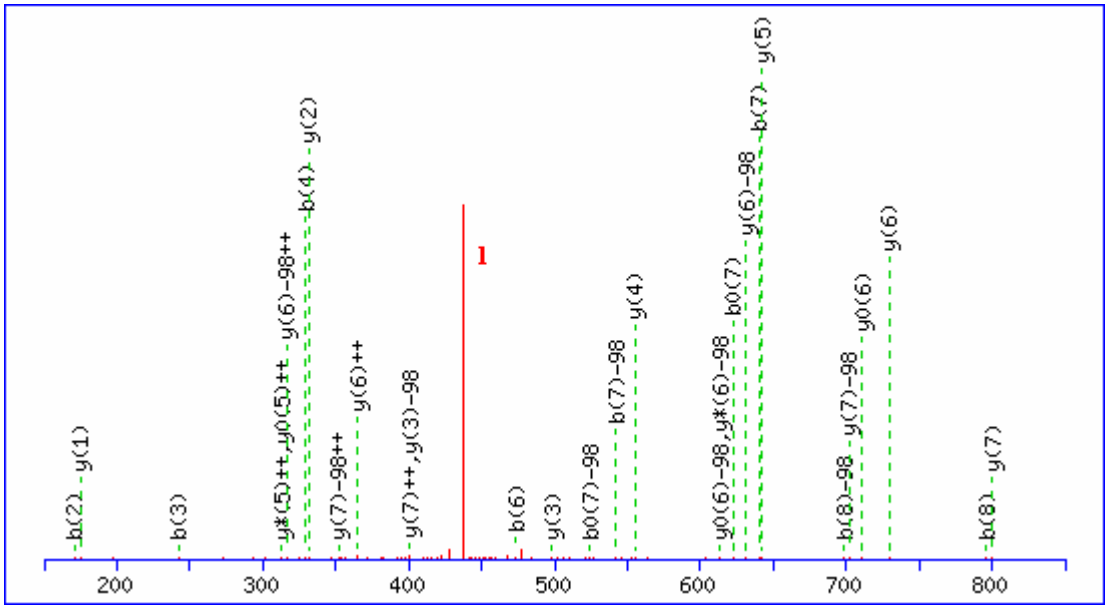
MS/MS Fragmentation of **GLASSGpSR**



Monoisotopic mass of neutral peptide Mr(calc): 813.3382
Variable modifications:
S7 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 41 **Expect:** 0.0043
Matches (Bold Red): 12/102 fragment ions using 12 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180			G							8
2	**171.1128**	86.0600			L	757.3240	379.1656	740.2975	370.6524	739.3134	370.1604	7
3	**242.1499**	121.5786			A	**644.2399**	**322.6236**	627.2134	314.1103	626.2294	313.6183	6
4	329.1819	165.0946	311.1714	156.0893	S	**573.2028**	287.1051	556.1763	278.5918	555.1923	278.0998	5
5	416.2140	208.6106	**398.2034**	199.6053	S	**486.1708**	243.5890	469.1442	235.0758	468.1602	234.5838	4
6	473.2354	237.1214	455.2249	228.1161	G	**399.1388**	200.0730	382.1122	191.5597	381.1282	191.0677	3
7	640.2338	320.6205	622.2232	311.6153	S	342.1173	**171.5623**	325.0908	163.0490	324.1067	162.5570	2
8					R	175.1190	88.0631	158.0924	79.5498			1

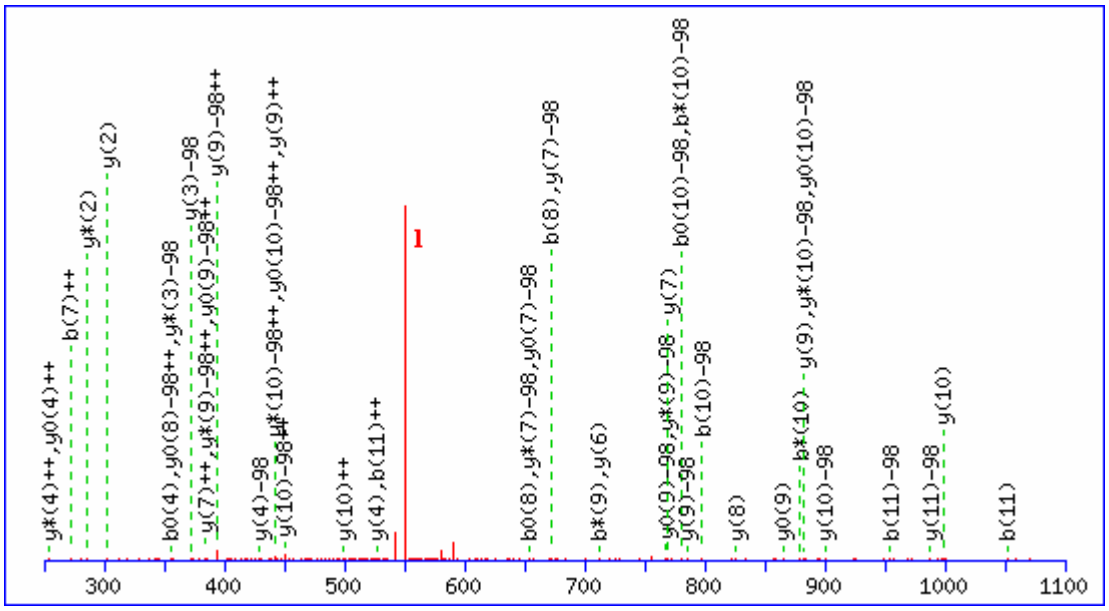
MS/MS Fragmentation of **GLASSGp*SRR**



Monoisotopic mass of neutral peptide Mr(calc): 969.4393
Variable modifications:
S7 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 42 **Expect:** 0.0025
Matches (Bold Red): 29/118 fragment ions using 37 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180					G							9
2	**171.1128**	86.0600					L	815.4482	408.2277	798.4217	399.7145	797.4376	399.2225	8
3	**242.1499**	121.5786					A	**702.3641**	**351.6857**	685.3376	343.1724	684.3536	342.6804	7
4	**329.1819**	165.0946			311.1714	156.0893	S	**631.3270**	**316.1672**	**614.3005**	307.6539	**613.3165**	307.1619	6
5	416.2140	208.6106			398.2034	199.6053	S	544.2950	272.6511	527.2685	264.1379	526.2844	263.6459	5
6	**473.2354**	237.1214			455.2249	228.1161	G	457.2630	229.1351	440.2364	220.6219	439.2524	220.1298	4
7	**542.2569**	271.6321			**524.2463**	262.6268	S	**400.2415**	200.6244	383.2150	192.1111	382.2310	191.6191	3
8	**698.3580**	349.6826	681.3314	341.1694	680.3474	340.6774	R	**331.2201**	166.1137	314.1935	157.6004			2
9							R	**175.1190**	88.0631	158.0924	79.5498			1

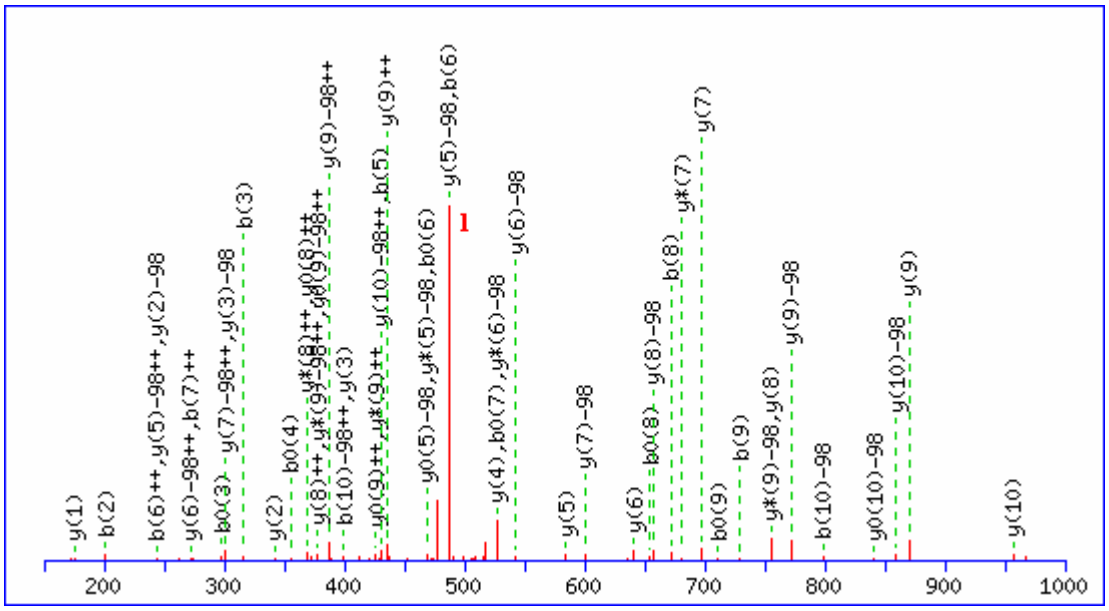
MS/MS Fragmentation of **LSDGGGGQGpSRK**



Monoisotopic mass of neutral peptide Mr(calc): 1197.5139
Variable modifications:
S10 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 28 **Expect:** 0.045
Matches (Bold Red): 46/178 fragment ions using 72 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							12
2	201.1234	101.0653			183.1128	92.0600	S	**987.4602**	494.2337	970.4337	485.7205	969.4497	485.2285	11
3	316.1503	158.5788			298.1397	149.5735	D	**900.4282**	**450.7177**	**883.4016**	**442.2045**	**882.4176**	**441.7124**	10
4	373.1718	187.0895			**355.1612**	178.0842	G	**785.4012**	**393.2043**	**768.3747**	**384.6910**	**767.3907**	**384.1990**	9
5	430.1932	215.6003			412.1827	206.5950	G	728.3798	364.6935	711.3532	356.1803	710.3692	**355.6882**	8
6	487.2147	244.1110			469.2041	235.1057	G	**671.3583**	336.1828	**654.3318**	327.6695	**653.3478**	327.1775	7
7	544.2361	**272.6217**			526.2256	263.6164	G	614.3369	307.6721	597.3103	299.1588	596.3263	298.6668	6
8	**672.2947**	336.6510	655.2682	328.1377	**654.2842**	327.6457	Q	557.3154	279.1613	540.2889	270.6481	539.3048	270.1561	5
9	729.3162	365.1617	**712.2896**	356.6485	711.3056	356.1564	G	**429.2568**	215.1321	412.2303	206.6188	411.2463	206.1268	4
10	**798.3376**	399.6725	**781.3111**	391.1592	**780.3271**	390.6672	S	**372.2354**	186.6213	**355.2088**	178.1080	354.2248	177.6160	3
11	**954.4388**	477.7230	937.4122	469.2097	936.4282	468.7177	R	**303.2139**	152.1106	**286.1874**	143.5973			2
12							K	147.1128	74.0600	130.0863	65.5468			1

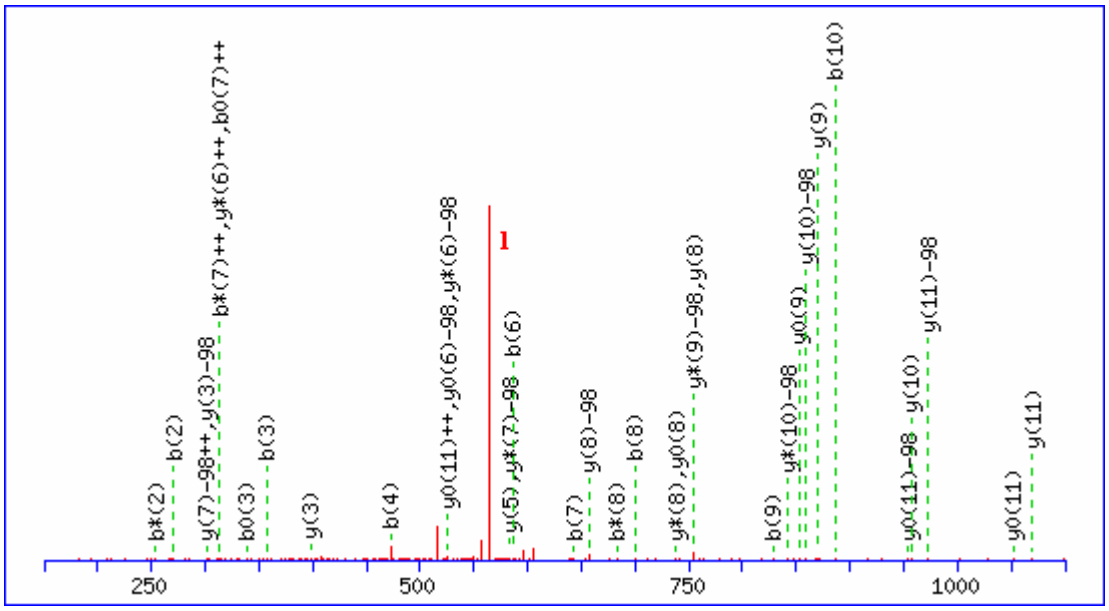
MS/MS Fragmentation of **LSDGGGGQGpSR**



Monoisotopic mass of neutral peptide Mr(calc): 1069.4189
Variable modifications:
S10 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 76 **Expect:** 5.3e-07
Matches (Bold Red): 53/162 fragment ions using 48 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							11
2	**201.1234**	101.0653			183.1128	92.0600	S	**957.3422**	479.1747	940.3156	470.6614	939.3316	470.1694	10
3	**316.1503**	158.5788			**298.1397**	149.5735	D	**870.3101**	**435.6587**	853.2836	**427.1454**	852.2996	**426.6534**	9
4	373.1718	187.0895			**355.1612**	178.0842	G	**755.2832**	**378.1452**	738.2566	**369.6320**	737.2726	**369.1399**	8
5	**430.1932**	215.6003			412.1827	206.5950	G	**698.2617**	349.6345	**681.2352**	341.1212	680.2512	340.6292	7
6	**487.2147**	**244.1110**			**469.2041**	235.1057	G	**641.2403**	321.1238	624.2137	312.6105	623.2297	312.1185	6
7	544.2361	**272.6217**			**526.2256**	263.6164	G	**584.2188**	292.6130	567.1923	284.0998	566.2082	283.6078	5
8	**672.2947**	336.6510	655.2682	328.1377	**654.2842**	327.6457	Q	**527.1973**	264.1023	510.1708	255.5890	509.1868	255.0970	4
9	**729.3162**	365.1617	712.2896	356.6485	**711.3056**	356.1564	G	**399.1388**	200.0730	382.1122	191.5597	381.1282	191.0677	3
10	896.3145	448.6609	879.2880	440.1476	878.3040	439.6556	S	**342.1173**	171.5623	325.0908	163.0490	324.1067	162.5570	2
11							R	**175.1190**	88.0631	158.0924	79.5498			1

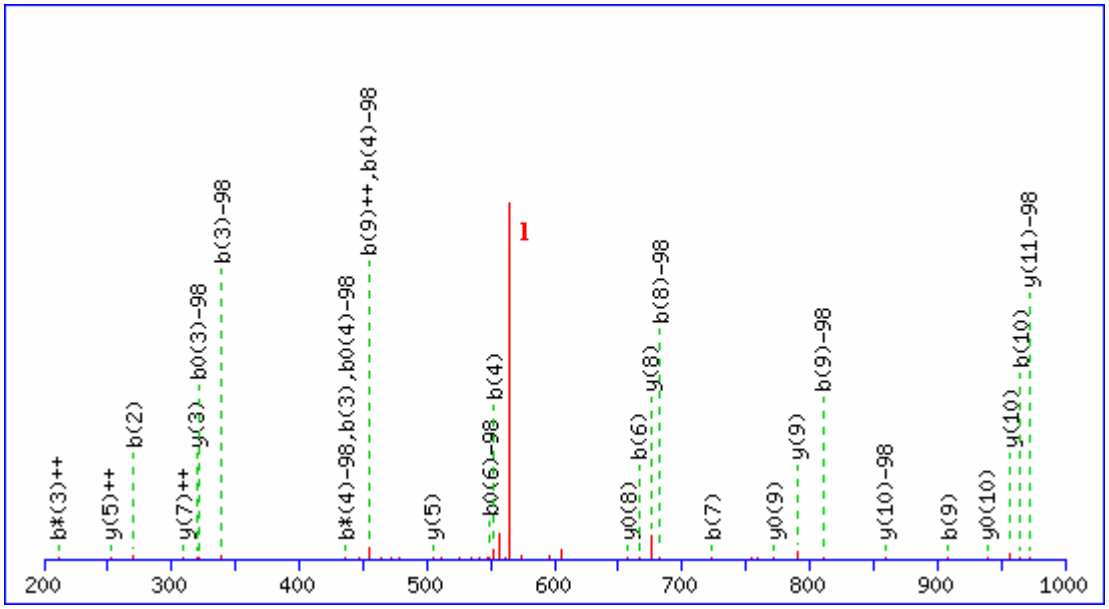
MS/MS Fragmentation of **RLSDGGGGQGpSR**



Monoisotopic mass of neutral peptide Mr(calc): 1225.5200
Variable modifications:
S11 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 49 **Expect:** 0.00061
Matches (Bold Red): 39/192 fragment ions using 63 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	157.1084	79.0578	140.0818	70.5446			R							12
2	**270.1924**	135.5999	**253.1659**	127.0866			L	**972.4493**	486.7283	955.4228	478.2150	**954.4388**	477.7230	11
3	**357.2245**	179.1159	340.1979	170.6026	**339.2139**	170.1106	S	**859.3653**	430.1863	**842.3387**	421.6730	841.3547	421.1810	10
4	**472.2514**	236.6293	455.2249	228.1161	454.2409	227.6241	D	772.3332	386.6703	**755.3067**	378.1570	754.3227	377.6650	9
5	529.2729	265.1401	512.2463	256.6268	511.2623	256.1348	G	**657.3063**	329.1568	640.2797	320.6435	639.2957	320.1515	8
6	**586.2943**	293.6508	569.2678	285.1375	568.2838	284.6455	G	600.2848	**300.6461**	**583.2583**	292.1328	582.2743	291.6408	7
7	**643.3158**	322.1615	626.2892	**313.6483**	625.3052	**313.1563**	G	543.2634	272.1353	**526.2368**	263.6220	**525.2528**	263.1300	6
8	**700.3373**	350.6723	**683.3107**	342.1590	682.3267	341.6670	G	486.2419	243.6246	469.2154	235.1113	468.2313	234.6193	5
9	**828.3958**	414.7016	811.3693	406.1883	810.3853	405.6963	Q	429.2204	215.1139	412.1939	206.6006	411.2099	206.1086	4
10	**885.4173**	443.2123	868.3907	434.6990	867.4067	434.2070	G	**301.1619**	151.0846	284.1353	142.5713	283.1513	142.0793	3
11	**954.4388**	477.7230	937.4122	469.2097	936.4282	468.7177	S	244.1404	122.5738	227.1139	114.0606	226.1298	113.5686	2
12							R	175.1190	88.0631	158.0924	79.5498			1

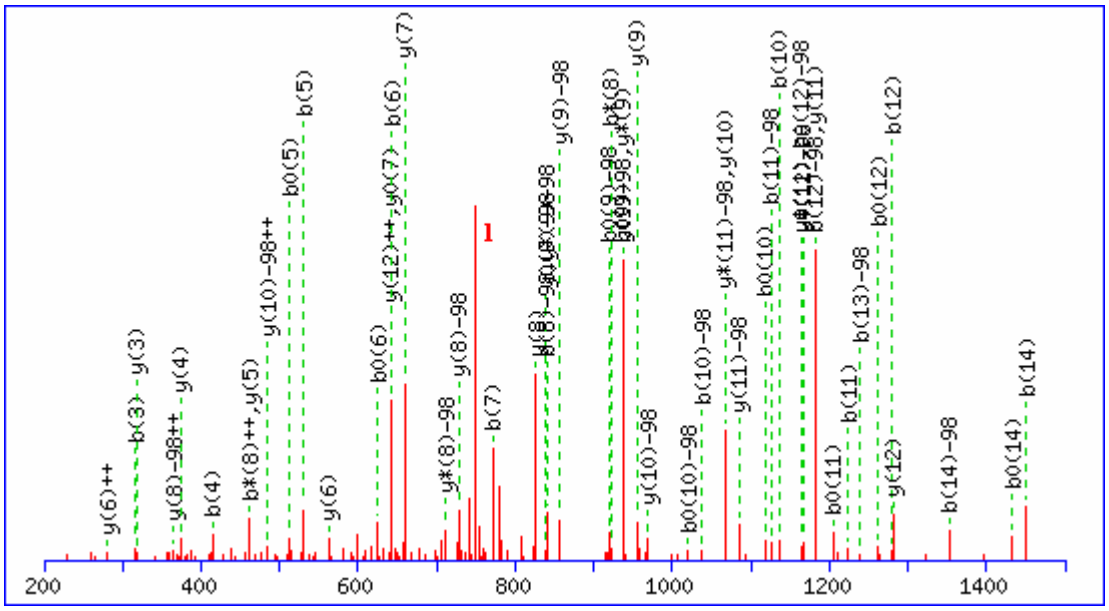
MS/MS Fragmentation of **RLpSDGGGGQGSR**



Monoisotopic mass of neutral peptide Mr(calc): 1225.5200
Variable modifications:
S3 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 58 **Expect:** 7.1e-05
Matches (Bold Red): 29/192 fragment ions using 34 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	157.1084	79.0578	140.0818	70.5446			R							12
2	**270.1924**	135.5999	253.1659	127.0866			L	**972.4493**	486.7283	955.4228	478.2150	954.4388	477.7230	11
3	**339.2139**	170.1106	322.1874	161.5973	**321.2033**	161.1053	S	**859.3653**	430.1863	842.3387	421.6730	841.3547	421.1810	10
4	**454.2408**	227.6241	**437.2143**	219.1108	**436.2303**	218.6188	D	**790.3438**	395.6755	773.3173	387.1623	**772.3332**	386.6703	9
5	511.2623	256.1348	494.2358	247.6215	493.2517	247.1295	G	**675.3169**	338.1621	658.2903	329.6488	**657.3063**	329.1568	8
6	568.2838	284.6455	551.2572	276.1322	**550.2732**	275.6402	G	618.2954	**309.6513**	601.2689	301.1381	600.2848	300.6461	7
7	625.3052	313.1563	608.2787	304.6430	607.2947	304.1510	G	561.2739	281.1406	544.2474	272.6273	543.2634	272.1353	6
8	**682.3267**	341.6670	665.3001	333.1537	664.3161	332.6617	G	**504.2525**	**252.6299**	487.2259	244.1166	486.2419	243.6246	5
9	**810.3853**	405.6963	793.3587	397.1830	792.3747	396.6910	Q	447.2310	224.1191	430.2045	215.6059	429.2205	215.1139	4
10	867.4067	434.2070	850.3802	425.6937	849.3962	425.2017	G	**319.1724**	160.0899	302.1459	151.5766	301.1619	151.0846	3
11	954.4388	477.7230	937.4122	469.2097	936.4282	468.7177	S	262.1510	131.5791	245.1244	123.0659	244.1404	122.5738	2
12							R	175.1190	88.0631	158.0924	79.5498			1

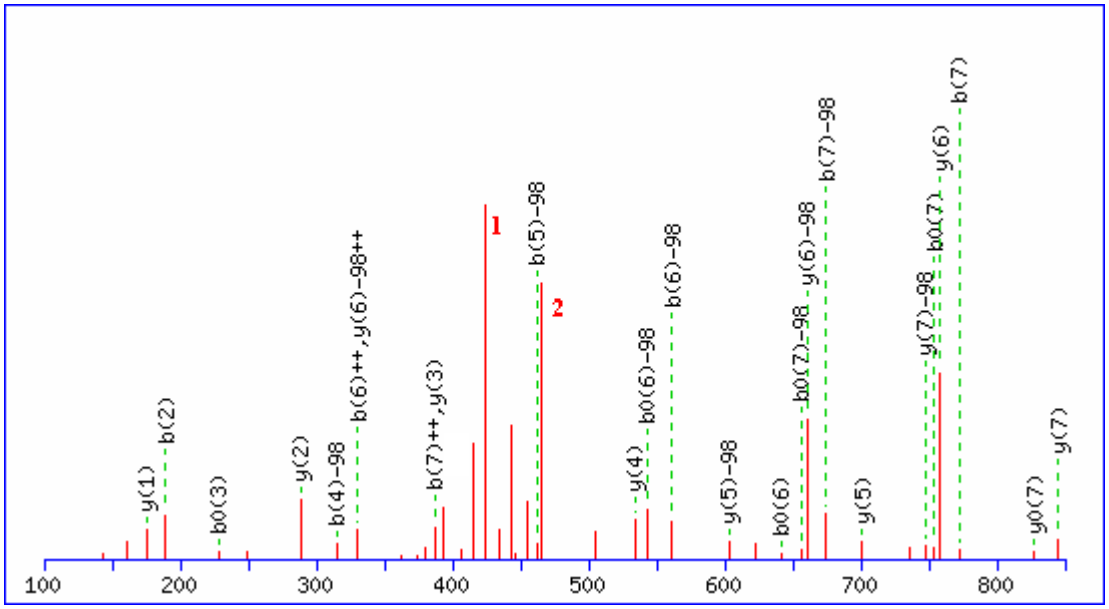
MS/MS Fragmentation of **GEEVDIQpSPTSGGIK**



Monoisotopic mass of neutral peptide Mr(calc): 1595.7080
Variable modifications:
S8 : Phospho (ST), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 94 **Expect:** 2.1e-08
Matches (Bold Red): 54/230 fragment ions using 63 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	58.0287	29.5180					G							15
2	187.0713	94.0393			169.0608	85.0340	E	1441.7169	721.3621	1424.6904	712.8488	1423.7063	712.3568	14
3	**316.1139**	158.5606			298.1034	149.5553	E	1312.6743	656.8408	1295.6478	648.3275	1294.6637	647.8355	13
4	**415.1823**	208.0948			397.1718	199.0895	V	1183.6317	592.3195	**1166.6052**	583.8062	1165.6212	583.3142	12
5	**530.2093**	265.6083			**512.1987**	256.6030	D	**1084.5633**	542.7853	**1067.5368**	534.2720	1066.5527	533.7800	11
6	**643.2933**	322.1503			**625.2828**	313.1450	I	**969.5364**	**485.2718**	952.5098	476.7585	951.5258	476.2665	10
7	**771.3519**	386.1796	754.3254	377.6663	753.3414	377.1743	Q	**856.4523**	428.7298	**839.4258**	420.2165	**838.4417**	419.7245	9
8	**840.3734**	420.6903	823.3468	412.1771	822.3628	411.6850	S	**728.3937**	**364.7005**	**711.3672**	356.1872	710.3832	355.6952	8
9	**937.4261**	469.2167	920.3996	460.7034	**919.4156**	460.2114	P	**659.3723**	330.1898	642.3457	321.6765	**641.3617**	321.1845	7
10	**1038.4738**	519.7406	1021.4473	511.2273	**1020.4633**	510.7353	T	**562.3195**	**281.6634**	545.2930	273.1501	544.3089	272.6581	6
11	**1125.5059**	563.2566	1108.4793	554.7433	1107.4953	554.2513	S	**461.2718**	231.1395	444.2453	222.6263	443.2613	222.1343	5
12	**1182.5273**	591.7673	1165.5008	583.2540	**1164.5168**	582.7620	G	**374.2398**	187.6235	357.2132	179.1103			4
13	**1239.5488**	620.2780	1222.5222	611.7648	1221.5382	611.2727	G	**317.2183**	159.1128	300.1918	150.5995			3
14	**1352.6328**	676.8201	1335.6063	668.3068	1334.6223	667.8148	I	260.1969	130.6021	243.1703	122.0888			2
15							K	147.1128	74.0600	130.0863	65.5468			1

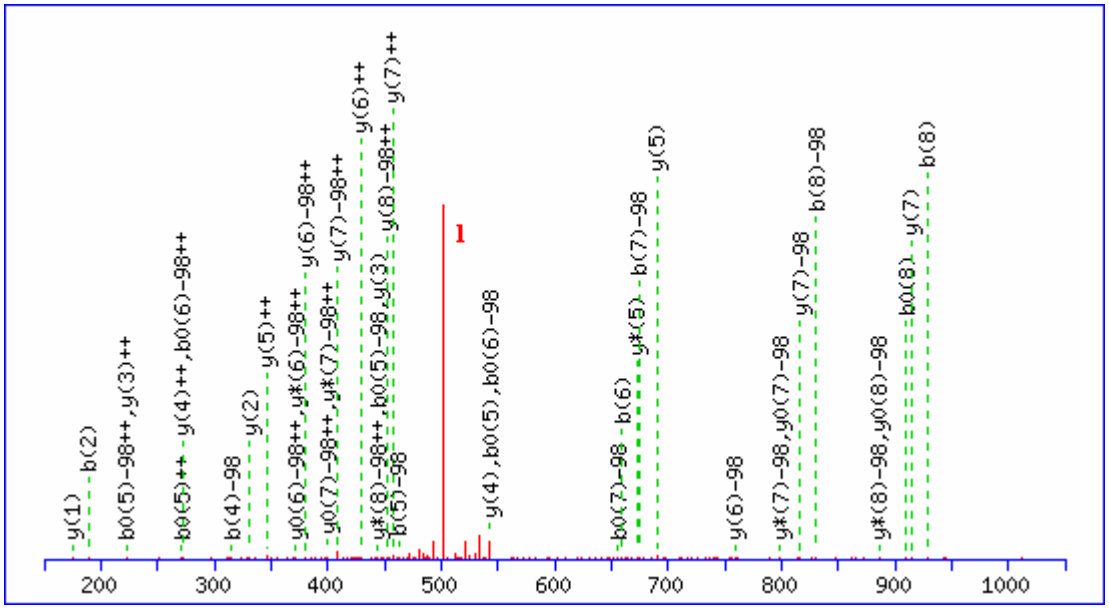
MS/MS Fragmentation of **TSGpSFVLR**



Monoisotopic mass of neutral peptide Mr(calc): 945.4321
Variable modifications:
S4 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 44 **Expect:** 0.0019
Matches (Bold Red): 25/96 fragment ions using 35 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	102.0550	51.5311	84.0444	42.5258	T							8
2	**189.0870**	95.0471	171.0764	86.0418	S	**747.4148**	374.2110	730.3882	365.6978	729.4042	365.2058	7
3	246.1084	123.5579	**228.0979**	114.5526	G	**660.3828**	**330.6950**	643.3562	322.1817	642.3722	321.6897	6
4	**315.1299**	158.0686	297.1193	149.0633	S	**603.3613**	302.1843	586.3347	293.6710	585.3507	293.1790	5
5	**462.1983**	231.6028	444.1877	222.5975	F	**534.3398**	267.6736	517.3133	259.1603			4
6	**561.2667**	281.1370	**543.2562**	272.1317	V	**387.2714**	194.1394	370.2449	185.6261			3
7	**674.3508**	337.6790	**656.3402**	328.6738	L	**288.2030**	144.6051	271.1765	136.0919			2
8					R	**175.1190**	88.0631	158.0924	79.5498			1

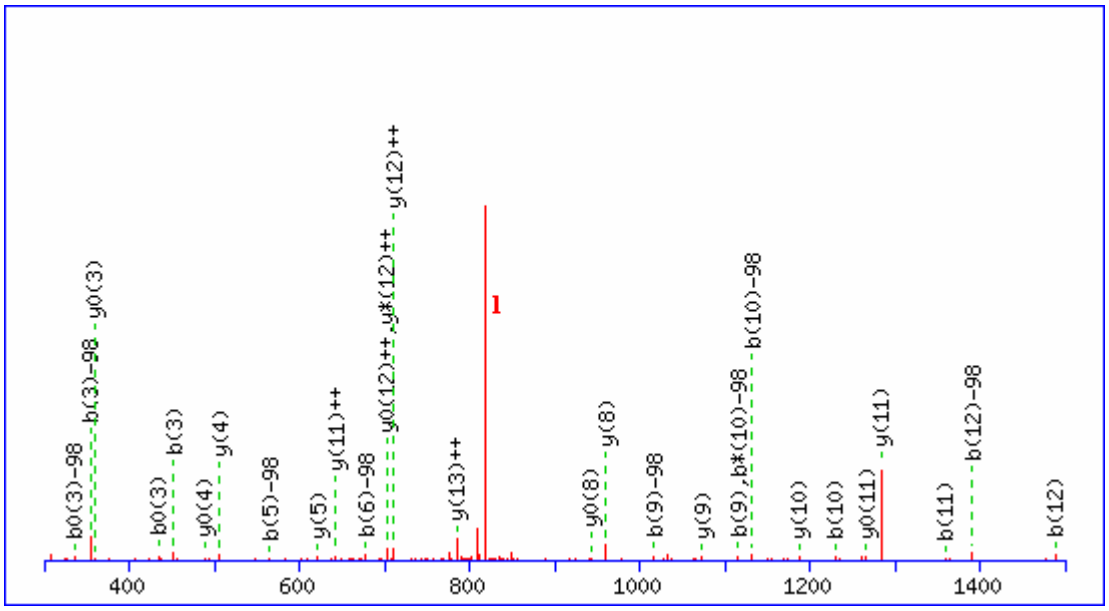
MS/MS Fragmentation of **TSGpSFVLRR**



Monoisotopic mass of neutral peptide Mr(calc): 1101.5332
Variable modifications:
S4 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 36 **Expect:** 0.0088
Matches (Bold Red): 41/112 fragment ions using 63 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	102.0550	51.5311			84.0444	42.5258	T							9
2	**189.0870**	95.0471			171.0764	86.0418	S	903.5159	**452.2616**	**886.4893**	**443.7483**	**885.5053**	443.2563	8
3	246.1084	123.5579			228.0979	114.5526	G	**816.4839**	**408.7456**	**799.4573**	**400.2323**	**798.4733**	**399.7403**	7
4	**315.1299**	158.0686			297.1193	149.0633	S	**759.4624**	**380.2348**	742.4358	**371.7216**	741.4518	**371.2296**	6
5	**462.1983**	231.6028			**444.1877**	**222.5975**	F	**690.4409**	**345.7241**	**673.4144**	337.2108			5
6	561.2667	281.1370			**543.2562**	**272.1317**	V	**543.3725**	**272.1899**	526.3460	263.6766			4
7	**674.3508**	337.6790			**656.3402**	328.6737	L	**444.3041**	**222.6557**	427.2776	214.1424			3
8	**830.4519**	415.7296	813.4253	407.2163	812.4413	406.7243	R	**331.2201**	166.1137	314.1935	157.6004			2
9							R	**175.1190**	88.0631	158.0924	79.5498			1

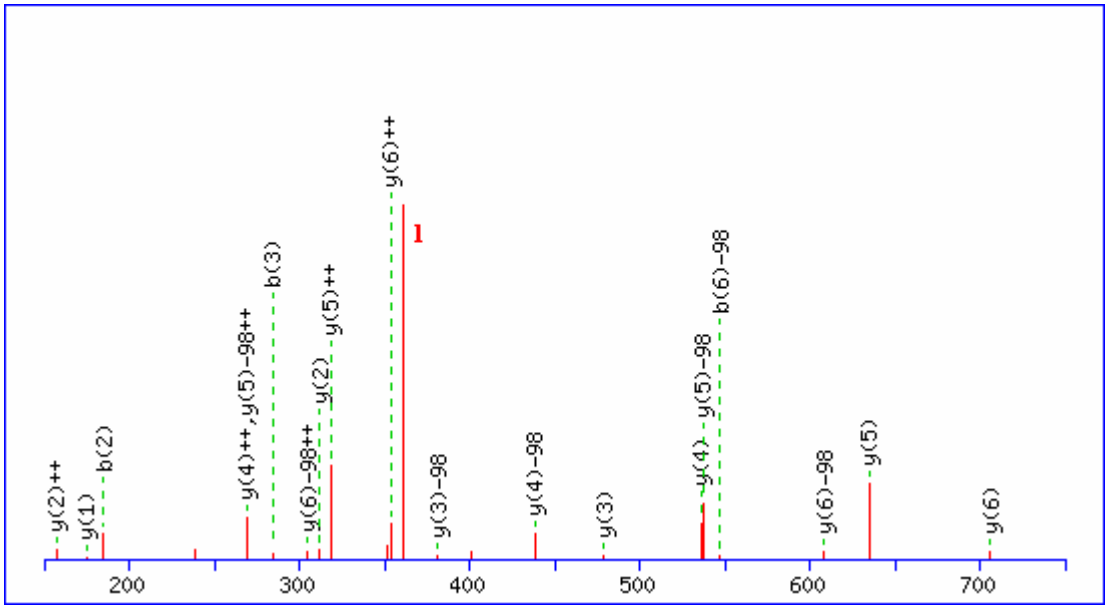
MS/MS Fragmentation of **pSFHPNLPLEDEETK**



Monoisotopic mass of neutral peptide Mr(calc): 1734.7502
Variable modifications:
S1 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 50 **Expect:** 0.00043
Matches (Bold Red): 29/216 fragment ions using 39 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	70.0287	35.5180			52.0182	26.5127	S							14
2	217.0971	109.0522			199.0866	100.0469	F	1568.7591	**784.8832**	1551.7325	776.3699	1550.7485	775.8779	13
3	**354.1561**	177.5817			**336.1455**	168.5764	H	1421.6907	**711.3490**	1404.6641	**702.8357**	1403.6801	**702.3437**	12
4	451.2088	226.1080			433.1982	217.1028	P	**1284.6318**	**642.8195**	1267.6052	634.3062	**1266.6212**	633.8142	11
5	**565.2517**	283.1295	548.2252	274.6162	547.2412	274.1242	N	**1187.5790**	594.2931	1170.5525	585.7799	1169.5684	585.2879	10
6	**678.3358**	339.6715	661.3093	331.1583	660.3252	330.6663	L	**1073.5361**	537.2717	1056.5095	528.7584	1055.5255	528.2664	9
7	775.3886	388.1979	758.3620	379.6846	757.3780	379.1926	P	**960.4520**	480.7296	943.4255	472.2164	**942.4414**	471.7244	8
8	888.4726	444.7400	871.4461	436.2267	870.4621	435.7347	L	863.3993	432.2033	846.3727	423.6900	845.3887	423.1980	7
9	**1017.5152**	509.2612	1000.4887	500.7480	999.5046	500.2560	E	750.3152	375.6612	733.2886	367.1480	732.3046	366.6560	6
10	**1132.5422**	566.7747	**1115.5156**	558.2614	1114.5316	557.7694	D	**621.2726**	311.1399	604.2461	302.6267	603.2620	302.1347	5
11	1261.5847	631.2960	1244.5582	622.7827	1243.5742	622.2907	E	**506.2457**	253.6265	489.2191	245.1132	**488.2351**	244.6212	4
12	**1390.6273**	695.8173	1373.6008	687.3040	1372.6168	686.8120	E	377.2031	189.1052	360.1765	180.5919	**359.1925**	180.0999	3
13	1491.6750	746.3411	1474.6485	737.8279	1473.6644	737.3359	T	248.1605	124.5839	231.1339	116.0706	230.1499	115.5786	2
14							K	147.1128	74.0600	130.0863	65.5468			1

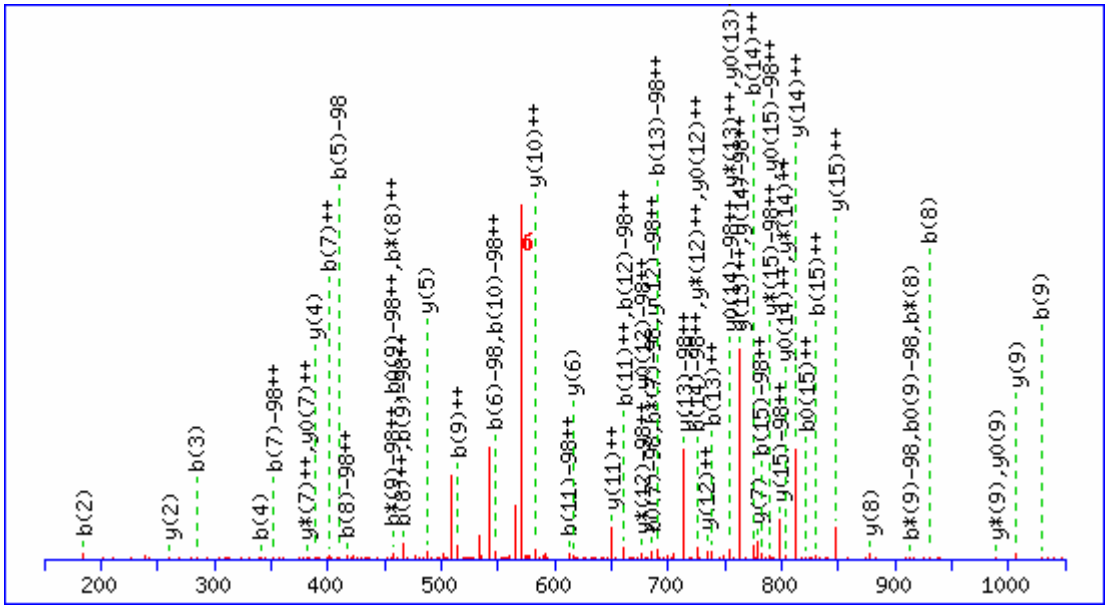
MS/MS Fragmentation of **IAVGpSHR**



Monoisotopic mass of neutral peptide Mr(calc): 818.3800
Variable modifications:
S5 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 65 **Expect:** 1.1e-05
Matches (Bold Red): 19/80 fragment ions using 22 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493			I							7
2	**185.1284**	93.0679			A	**608.3263**	**304.6668**	591.2998	296.1535	590.3157	295.6615	6
3	**284.1969**	142.6021			V	**537.2892**	**269.1482**	520.2626	260.6350	519.2786	260.1430	5
4	341.2183	171.1128			G	**438.2208**	219.6140	421.1942	211.1008	420.2102	210.6087	4
5	410.2398	205.6235	392.2292	196.6182	S	**381.1993**	191.1033	364.1728	182.5900	363.1888	182.0980	3
6	**547.2987**	274.1530	529.2881	265.1477	H	**312.1779**	**156.5926**	295.1513	148.0793			2
7					R	**175.1190**	88.0631	158.0924	79.5498			1

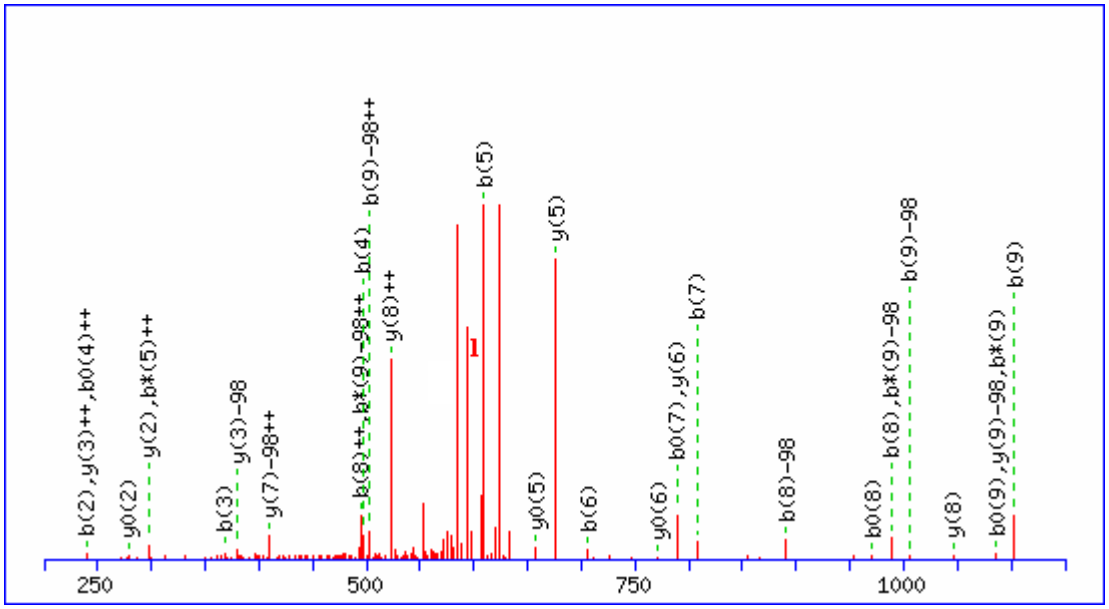
MS/MS Fragmentation of **IAVGpSHREVYEVGALK**



Monoisotopic mass of neutral peptide Mr(calc): 1806.9029
Variable modifications:
S5 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 40 **Expect:** 0.0052
Matches (Bold Red): 66/236 fragment ions using 97 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					I							16
2	**185.1285**	93.0679					A	1694.8262	**847.9167**	1677.7996	839.4034	1676.8156	838.9114	15
3	**284.1969**	142.6021					V	1623.7890	**812.3982**	1606.7625	**803.8849**	1605.7785	**803.3929**	14
4	**341.2183**	171.1128					G	1524.7206	**762.8640**	1507.6941	**754.3507**	1506.7101	**753.8587**	13
5	508.2167	254.6120			490.2061	245.6067	S	1467.6992	**734.3532**	1450.6726	**725.8399**	1449.6886	**725.3479**	12
6	645.2756	323.1414			627.2650	314.1362	H	1300.7008	**650.8540**	1283.6743	642.3408	1282.6902	641.8488	11
7	801.3767	**401.1920**	784.3502	392.6787	783.3661	392.1867	R	**1163.6419**	**582.3246**	1146.6154	573.8113	1145.6313	573.3193	10
8	**930.4193**	**465.7133**	**913.3928**	**457.2000**	912.4087	456.7080	E	**1007.5408**	504.2740	**990.5142**	495.7608	**989.5302**	495.2687	9
9	**1029.4877**	**515.2475**	1012.4612	506.7342	1011.4771	506.2422	V	**878.4982**	439.7527	861.4716	431.2395	860.4876	430.7475	8
10	1192.5510	596.7792	1175.5245	588.2659	1174.5405	587.7739	Y	**779.4298**	390.2185	762.4032	**381.7053**	761.4192	**381.2132**	7
11	1321.5936	**661.3005**	1304.5671	652.7872	1303.5831	652.2952	E	**616.3665**	308.6869	599.3399	300.1736	598.3559	299.6816	6
12	1420.6620	710.8347	1403.6355	702.3214	1402.6515	701.8294	V	**487.3239**	244.1656	470.2973	235.6523			5
13	1477.6835	**739.3454**	1460.6570	730.8321	1459.6729	730.3401	G	**388.2554**	194.6314	371.2289	186.1181			4
14	1548.7206	**774.8640**	1531.6941	766.3507	1530.7101	765.8587	A	331.2340	166.1206	314.2074	157.6074			3
15	1661.8047	**831.4060**	1644.7781	822.8927	1643.7941	**822.4007**	L	**260.1969**	130.6021	243.1703	122.0888			2
16							K	147.1128	74.0600	130.0863	65.5468			1

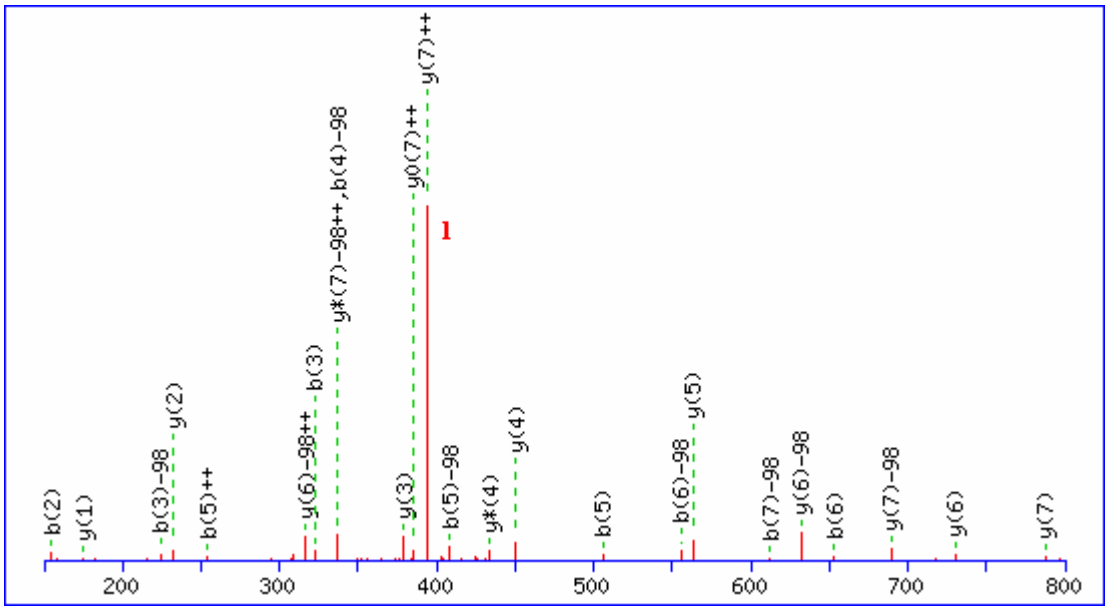
MS/MS Fragmentation of **THEQLPTp*TDY**



Monoisotopic mass of neutral peptide Mr(calc): 1283.5071
Variable modifications:
T8 : Phospho (T), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 53 **Expect:** 0.00014
Matches (Bold Red): 32/134 fragment ions using 35 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	102.0550	51.5311			84.0444	42.5258	T							10
2	**239.1139**	120.0606			221.1033	111.0553	H	**1085.4898**	543.2485	1068.4633	534.7353	1067.4792	534.2433	9
3	**368.1565**	184.5819			350.1459	175.5766	E	948.4309	474.7191	931.4043	466.2058	930.4203	465.7138	8
4	**496.2150**	248.6112	479.1885	240.0979	478.2045	**239.6059**	Q	819.3883	**410.1978**	802.3618	401.6845	801.3777	401.1925	7
5	**609.2991**	305.1532	592.2725	**296.6399**	591.2885	296.1479	L	691.3297	346.1685			673.3192	337.1632	6
6	**706.3519**	353.6796	689.3253	345.1663	688.3413	344.6743	P	578.2457	289.6265			560.2351	280.6212	5
7	**807.3995**	404.2034	790.3730	395.6901	**789.3890**	395.1981	T	481.1929	241.1001			463.1823	232.0948	4
8	**890.4366**	445.7220	873.4101	437.2087	872.4261	436.7167	T	**380.1452**	190.5762			362.1347	181.5710	3
9	**1005.4636**	**503.2354**	**988.4370**	**494.7222**	987.4530	494.2301	D	**297.1081**	149.0577			**279.0975**	140.0524	2
10							Y	182.0812	91.5442					1

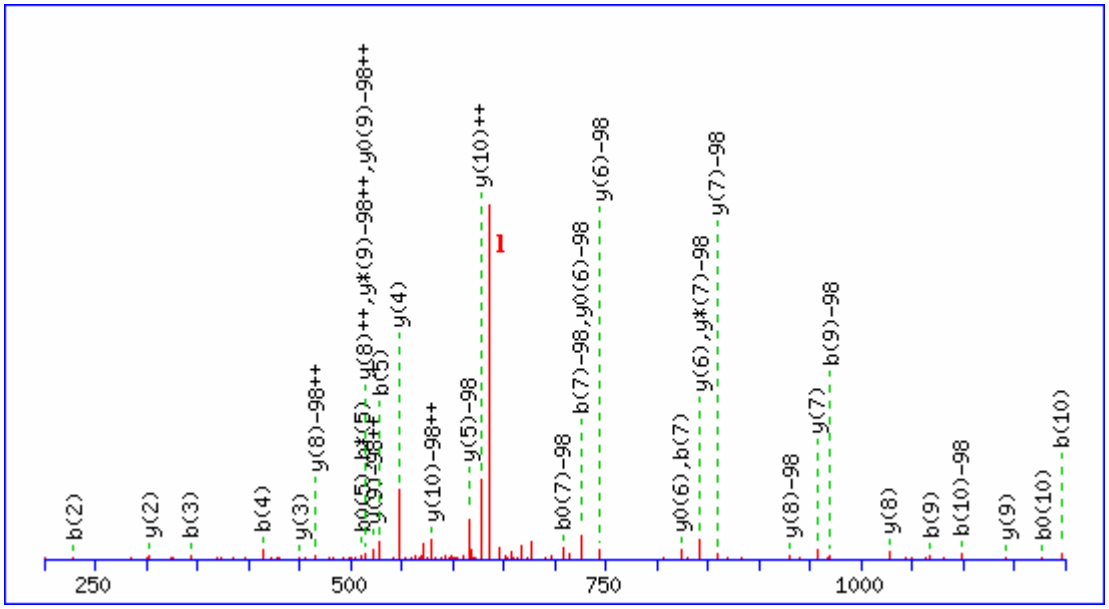
MS/MS Fragmentation of **PGpSIAFGR**



Monoisotopic mass of neutral peptide Mr(calc): 883.3953
Variable modifications:
S3 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 64 **Expect:** 1.8e-05
Matches (Bold Red): 24/88 fragment ions using 29 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	98.0600	49.5337			P							8
2	**155.0815**	78.0444			G	**787.3498**	**394.1786**	770.3233	385.6653	769.3393	**385.1733**	7
3	**322.0799**	161.5436	304.0693	152.5383	S	**730.3284**	365.6678	713.3018	357.1545	712.3178	356.6625	6
4	435.1639	218.0856	417.1534	209.0803	I	**563.3300**	282.1686	546.3035	273.6554			5
5	**506.2010**	**253.6042**	488.1905	244.5989	A	**450.2459**	225.6266	**433.2194**	217.1133			4
6	**653.2694**	327.1384	635.2589	318.1331	F	**379.2088**	190.1081	362.1823	181.5948			3
7	710.2909	355.6491	692.2803	346.6438	G	**232.1404**	116.5738	215.1139	108.0606			2
8					R	**175.1190**	88.0631	158.0924	79.5498			1

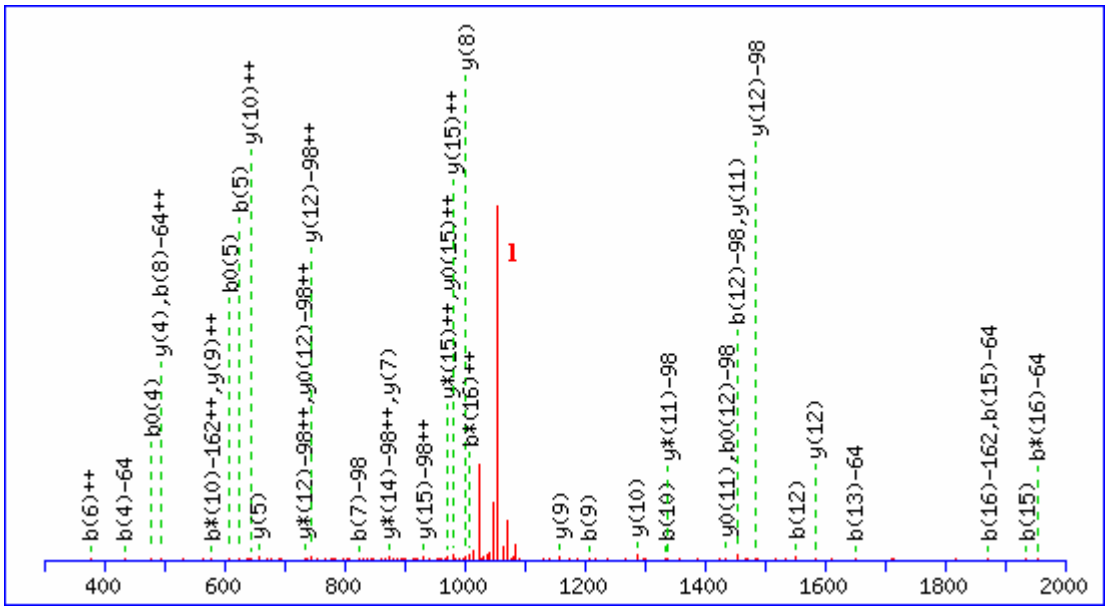
MS/MS Fragmentation of **DLNADKpSPFQR**



Monoisotopic mass of neutral peptide Mr(calc): 1369.6027
Variable modifications:
S7 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 63 **Expect:** 2.5e-05
Matches (Bold Red): 35/168 fragment ions using 35 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	116.0342	58.5207			98.0237	49.5155	D							11
2	**229.1183**	115.0628			211.1077	106.0575	L	1157.6062	**579.3067**	1140.5796	570.7934	1139.5956	570.3014	10
3	**343.1612**	172.0842	326.1347	163.5710	325.1506	163.0790	N	1044.5221	**522.7647**	1027.4956	**514.2514**	1026.5115	**513.7594**	9
4	**414.1983**	207.6028	397.1718	199.0895	396.1878	198.5975	A	**930.4792**	**465.7432**	913.4526	457.2299	912.4686	456.7379	8
5	**529.2253**	265.1163	**512.1987**	256.6030	**511.2147**	256.1110	D	**859.4421**	430.2247	**842.4155**	421.7114	841.4315	421.2194	7
6	657.3202	329.1637	640.2937	320.6505	639.3097	320.1585	K	**744.4151**	372.7112	727.3886	364.1979	**726.4046**	363.7059	6
7	**726.3417**	363.6745	709.3151	355.1612	**708.3311**	354.6692	S	**616.3202**	308.6637	599.2936	300.1504	598.3096	299.6584	5
8	823.3944	412.2009	806.3679	403.6876	805.3839	403.1956	P	**547.2987**	274.1530	530.2722	265.6397			4
9	**970.4628**	485.7351	953.4363	477.2218	952.4523	476.7298	F	**450.2459**	225.6266	433.2194	217.1133			3
10	**1098.5214**	549.7644	1081.4949	541.2511	1080.5109	540.7591	Q	**303.1775**	152.0924	286.1510	143.5791			2
11							R	175.1190	88.0631	158.0924	79.5498			1

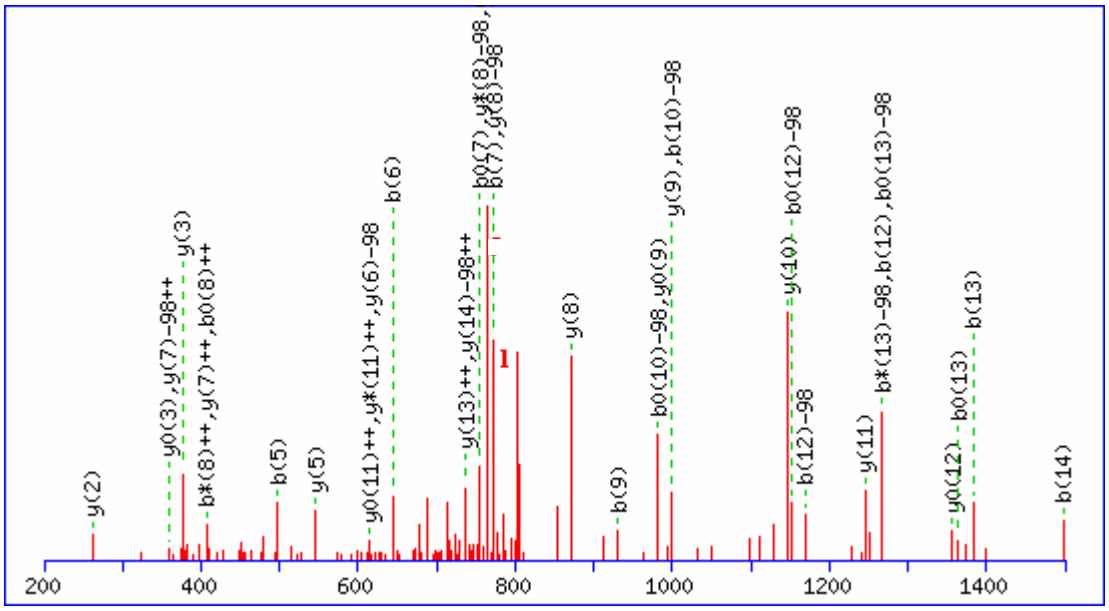
MS/MS Fragmentation of **MTTFEEp*SERESEYGYVR**



Monoisotopic mass of neutral peptide Mr(calc): 2207.8718
Variable modifications:
M1 : Oxidation (M), with neutral losses 63.9983(shown in table), 0.0000
S7 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 35 **Expect:** 0.0093
Matches (Bold Red): 40/390 fragment ions using 42 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	84.0444	42.5258					M							17
2	185.0921	93.0497			167.0815	84.0444	T	2061.8437	1031.4255	2044.8172	1022.9122	2043.8332	1022.4202	16
3	286.1398	143.5735			268.1292	134.5682	T	1960.7960	**980.9017**	1943.7695	**972.3884**	1942.7855	**971.8964**	15
4	**433.2082**	217.1077			415.1976	208.1024	F	1859.7484	930.3778	1842.7218	921.8645	1841.7378	921.3725	14
5	562.2508	281.6290			544.2402	272.6237	E	1712.6799	856.8436	1695.6534	848.3303	1694.6694	847.8383	13
6	691.2934	346.1503			673.2828	337.1450	E	**1583.6374**	792.3223	1566.6108	783.8090	1565.6268	783.3170	12
7	858.2917	429.6495			840.2811	420.6442	S	**1454.5948**	727.8010	1437.5682	719.2877	**1436.5842**	718.7957	11
8	987.3343	**494.1708**			969.3237	485.1655	E	**1287.5964**	**644.3018**	1270.5699	635.7886	1269.5858	635.2966	10
9	1143.4354	572.2213	1126.4089	563.7081	1125.4248	563.2161	R	**1158.5538**	**579.7805**	1141.5273	571.2673	1140.5432	570.7753	9
10	1272.4780	636.7426	1255.4515	628.2294	1254.4674	627.7374	E	**1002.4527**	501.7300	985.4262	493.2167	984.4421	492.7247	8
11	1359.5100	680.2587	1342.4835	671.7454	1341.4995	671.2534	S	**873.4101**	437.2087	856.3836	428.6954	855.3995	428.2034	7
12	1488.5526	744.7799	1471.5261	736.2667	1470.5421	735.7747	E	786.3781	393.6927	769.3515	385.1794	768.3675	384.6874	6
13	**1651.6160**	826.3116	1634.5894	817.7983	1633.6054	817.3063	Y	**657.3355**	329.1714	640.3089	320.6581			5
14	1708.6374	854.8223	1691.6109	846.3091	1690.6269	845.8171	G	**494.2722**	247.6397	477.2456	239.1264			4
15	**1871.7007**	936.3540	1854.6742	927.8407	1853.6902	927.3487	Y	437.2507	219.1290	420.2241	210.6157			3
16	1970.7692	985.8882	**1953.7426**	977.3749	1952.7586	976.8829	V	274.1874	137.5973	257.1608	129.0840			2
17							R	175.1190	88.0631	158.0924	79.5498			1

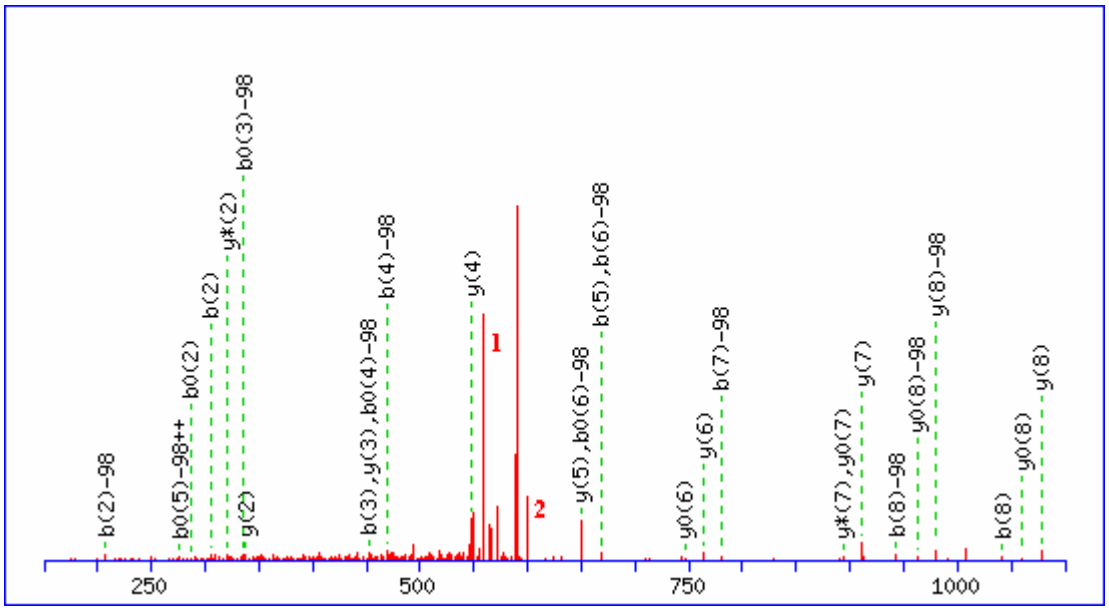
MS/MS Fragmentation of **AVVQVFEGTp*SGIDNK**



Monoisotopic mass of neutral peptide Mr(calc): 1642.7604
Variable modifications:
S10 : Phospho (ST), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 69 **Expect:** 7.1e-06
Matches (Bold Red): 37/230 fragment ions using 26 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	72.0444	36.5258					A							15
2	171.1128	86.0600					V	1572.7305	786.8689	1555.7040	778.3556	1554.7200	777.8636	14
3	270.1812	135.5942					V	1473.6621	**737.3347**	1456.6356	728.8214	1455.6515	728.3294	13
4	398.2398	199.6235	381.2132	191.1103			Q	1374.5937	687.8005	1357.5671	679.2872	**1356.5831**	678.7952	12
5	**497.3082**	249.1577	480.2817	240.6445			V	**1246.5351**	623.7712	1229.5086	**615.2579**	1228.5246	**614.7659**	11
6	**644.3766**	322.6920	627.3501	314.1787			F	**1147.4667**	574.2370	1130.4402	565.7237	1129.4561	565.2317	10
7	**773.4192**	387.2132	756.3927	378.7000	**755.4087**	378.2080	E	**1000.3983**	500.7028	983.3717	492.1895	**982.3877**	491.6975	9
8	830.4407	415.7240	813.4141	**407.2107**	812.4301	**406.7187**	G	**871.3557**	436.1815	854.3291	427.6682	853.3451	427.1762	8
9	**931.4884**	466.2478	914.4618	457.7345	913.4778	457.2425	T	814.3342	**407.6708**	797.3077	399.1575	796.3237	398.6655	7
10	1098.4867	549.7470	1081.4602	541.2337	1080.4761	540.7417	S	713.2866	357.1469	696.2600	348.6336	695.2760	348.1416	6
11	1155.5082	578.2577	1138.4816	569.7445	1137.4976	569.2524	G	**546.2882**	273.6477	529.2617	265.1345	528.2776	264.6425	5
12	**1268.5922**	634.7998	1251.5657	626.2865	1250.5817	625.7945	I	489.2667	245.1370	472.2402	236.6237	471.2562	236.1317	4
13	**1383.6192**	692.3132	1366.5926	683.8000	**1365.6086**	683.3079	D	**376.1827**	188.5950	359.1561	180.0817	**358.1721**	179.5897	3
14	**1497.6621**	749.3347	1480.6356	740.8214	1479.6515	740.3294	N	**261.1557**	131.0815	244.1292	122.5682			2
15							K	147.1128	74.0600	130.0863	65.5468			1

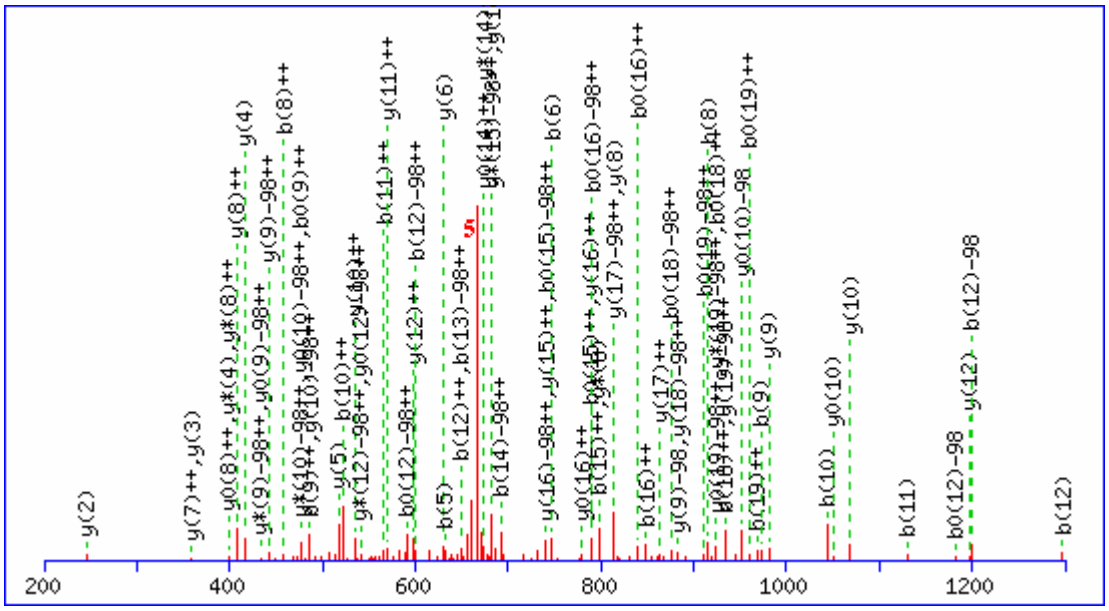
MS/MS Fragmentation of **HpSFDTPLYR**



Monoisotopic mass of neutral peptide Mr(calc): 1214.5121
Variable modifications:
S2 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 38 **Expect:** 0.0082
Matches (Bold Red): 28/104 fragment ions using 63 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	138.0662	69.5367			H							9
2	**305.0645**	153.0359	**287.0540**	144.0306	S	**1078.4605**	539.7339	1061.4339	531.2206	**1060.4499**	530.7286	8
3	**452.1330**	226.5701	434.1224	217.5648	F	**911.4621**	456.2347	**894.4356**	447.7214	**893.4516**	447.2294	7
4	567.1599	284.0836	549.1493	275.0783	D	**764.3937**	382.7005	747.3672	374.1872	**746.3832**	373.6952	6
5	**668.2076**	334.6074	650.1970	325.6021	T	**649.3668**	325.1870	632.3402	316.6738	631.3562	316.1817	5
6	765.2603	383.1338	747.2498	374.1285	P	**548.3191**	274.6632	531.2926	266.1499			4
7	878.3444	439.6758	860.3338	430.6706	L	**451.2663**	226.1368	434.2398	217.6235			3
8	**1041.4077**	521.2075	1023.3972	512.2022	Y	**338.1823**	169.5948	**321.1557**	161.0815			2
9					R	175.1190	88.0631	158.0924	79.5498			1

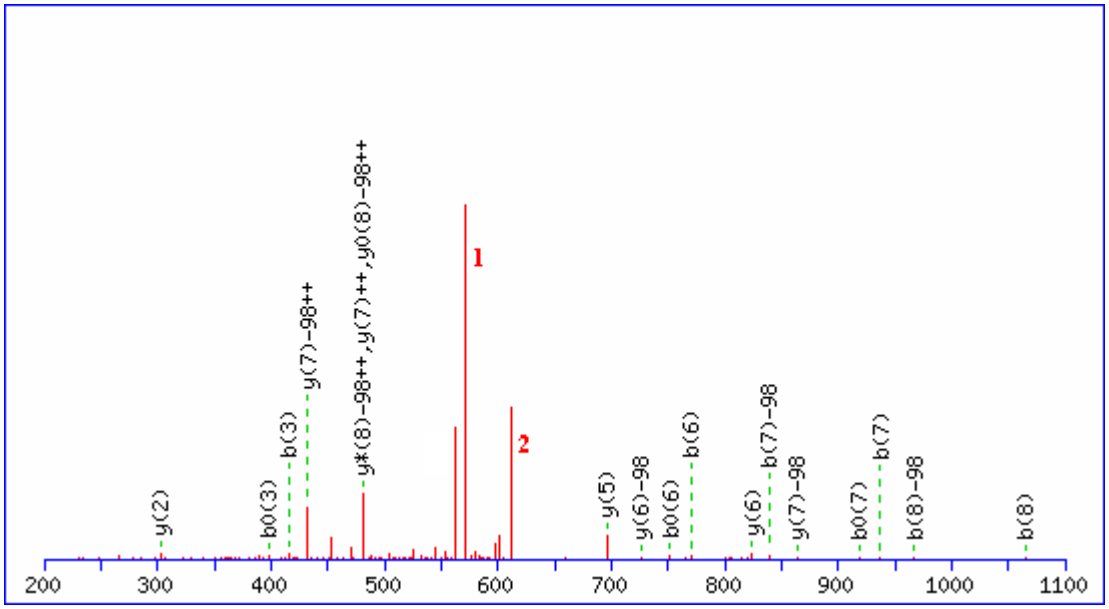
MS/MS Fragmentation of **FTHFVDPAGASp*SPSLTGLAR**



Monoisotopic mass of neutral peptide Mr(calc): 2109.9884
Variable modifications:
S12 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 78 **Expect:** 1.1e-06
Matches (Bold Red): 76/278 fragment ions using 61 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	148.0757	74.5415			F							20
2	249.1234	125.0653	231.1128	116.0600	T	1865.9504	**933.4788**	1848.9238	**924.9656**	1847.9398	**924.4735**	19
3	386.1823	193.5948	368.1717	184.5895	H	1764.9027	**882.9550**	1747.8762	874.4417	1746.8921	873.9497	18
4	533.2507	267.1290	515.2401	258.1237	F	1627.8438	**814.4255**	1610.8173	805.9123	1609.8332	805.4203	17
5	**632.3191**	316.6632	614.3085	307.6579	V	1480.7754	**740.8913**	1463.7488	732.3781	1462.7648	731.8860	16
6	**747.3460**	374.1767	729.3355	365.1714	D	1381.7070	691.3571	1364.6804	**682.8439**	1363.6964	**682.3518**	15
7	844.3988	422.7030	826.3882	413.6978	P	1266.6800	633.8437	1249.6535	625.3304	1248.6695	624.8384	14
8	**915.4359**	**458.2216**	897.4253	449.2163	A	1169.6273	585.3173	1152.6007	576.8040	1151.6167	576.3120	13
9	**972.4574**	**486.7323**	954.4468	**477.7270**	G	1098.5902	549.7987	1081.5636	**541.2854**	1080.5796	**540.7934**	12
10	**1043.4945**	**522.2509**	1025.4839	513.2456	A	1041.5687	521.2880	1024.5422	512.7747	1023.5581	512.2827	11
11	**1130.5265**	**565.7669**	1112.5159	556.7616	S	970.5316	**485.7694**	953.5050	**477.2562**	**952.5210**	**476.7642**	10
12	**1199.5480**	**600.2776**	**1181.5374**	**591.2723**	S	**883.4996**	**442.2534**	866.4730	**433.7401**	865.4890	**433.2481**	9
13	1296.6007	**648.8040**	1278.5902	639.7987	P	**814.4781**	**407.7427**	**797.4516**	**399.2294**	796.4675	**398.7374**	8
14	1383.6328	**692.3200**	1365.6222	**683.3147**	S	717.4254	**359.2163**	700.3988	350.7030	699.4148	350.2110	7
15	1496.7168	748.8620	1478.7062	**739.8568**	L	**630.3933**	315.7003	613.3668	307.1870	612.3828	306.6950	6
16	1597.7645	799.3859	1579.7539	**790.3806**	T	**517.3093**	259.1583	500.2827	250.6450	499.2987	250.1530	5
17	1654.7860	827.8966	1636.7754	818.8913	G	**416.2616**	208.6344	**399.2350**	200.1212			4
18	1767.8700	884.4386	1749.8594	**875.4334**	L	**359.2401**	180.1237	342.2136	171.6104			3
19	1838.9071	919.9572	1820.8966	**910.9519**	A	**246.1561**	123.5817	229.1295	115.0684			2
20					R	175.1190	88.0631	158.0924	79.5498			1

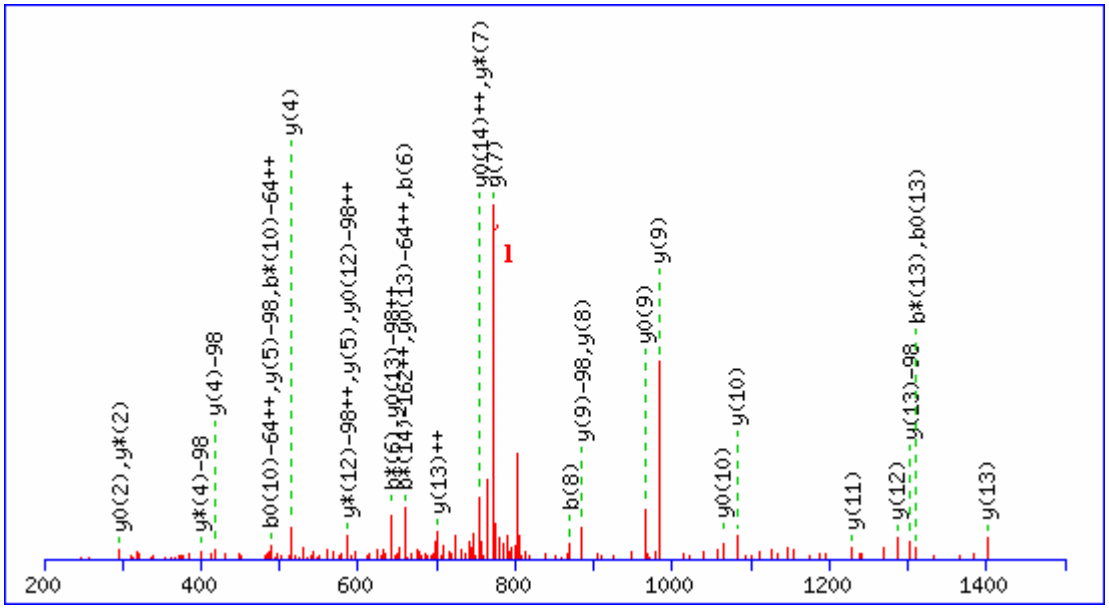
MS/MS Fragmentation of **YDHKPEpSQR**



Monoisotopic mass of neutral peptide Mr(calc): 1238.5081
Variable modifications:
S7 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 30 **Expect:** 0.02
Matches (Bold Red): 18/132 fragment ions using 20 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	164.0706	82.5389					Y							9
2	279.0975	140.0524			261.0870	131.0471	D	978.4751	489.7412	961.4486	**481.2279**	960.4646	**480.7359**	8
3	**416.1565**	208.5819			**398.1459**	199.5766	H	**863.4482**	**432.2277**	846.4217	423.7145	845.4376	423.2225	7
4	544.2514	272.6293	527.2249	264.1161	526.2409	263.6241	K	**726.3893**	363.6983	709.3628	355.1850	708.3787	354.6930	6
5	641.3042	321.1557	624.2776	312.6425	623.2936	312.1504	P	598.2943	299.6508	581.2678	291.1375	580.2838	290.6455	5
6	**770.3468**	385.6770	753.3202	377.1637	**752.3362**	376.6717	E	501.2416	251.1244	484.2150	242.6112	483.2310	242.1191	4
7	**839.3682**	420.1878	822.3417	411.6745	821.3577	411.1825	S	372.1990	186.6031	355.1724	178.0899	354.1884	177.5978	3
8	**967.4268**	484.2170	950.4003	475.7038	949.4162	475.2118	Q	**303.1775**	152.0924	286.1510	143.5791			2
9							R	175.1190	88.0631	158.0924	79.5498			1

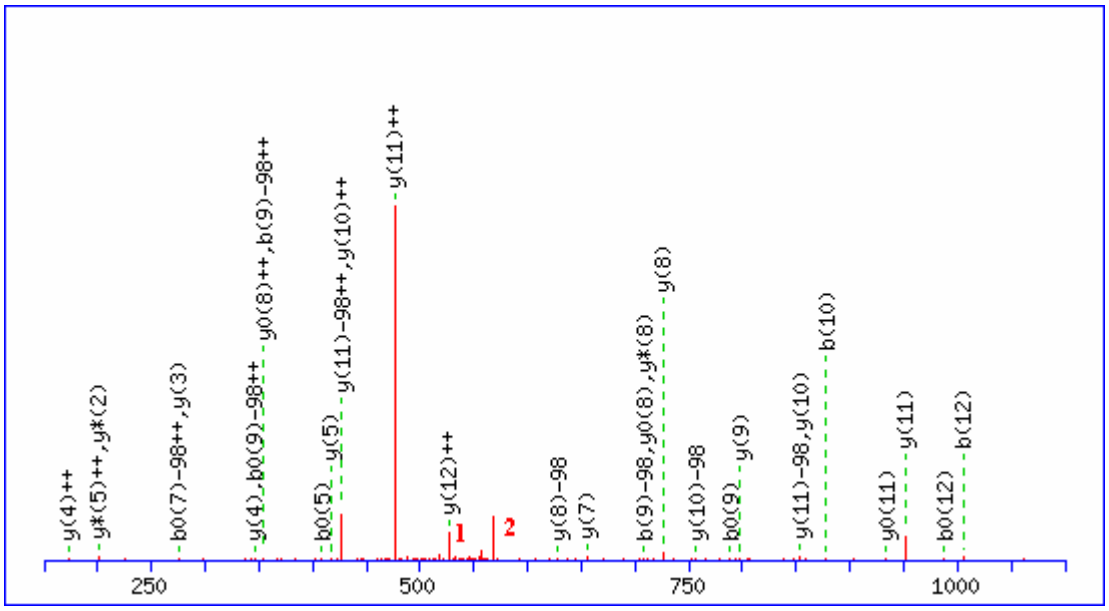
MS/MS Fragmentation of **LQNGMVPNGEASNp*SK**



Monoisotopic mass of neutral peptide Mr(calc): 1640.6865
Variable modifications:
M5 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
S14 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 75 **Expect:** 1.6e-06
Matches (Bold Red): 33/334 fragment ions using 29 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493					L							15
2	242.1499	121.5786	225.1234	113.0653			Q	1528.6098	764.8085	1511.5832	756.2952	1510.5992	**755.8032**	14
3	356.1928	178.6001	339.1663	170.0868			N	**1400.5512**	**700.7792**	1383.5246	692.2660	1382.5406	691.7739	13
4	413.2143	207.1108	396.1878	198.5975			G	**1286.5083**	643.7578	1269.4817	635.2445	1268.4977	634.7525	12
5	560.2497	280.6285	543.2232	272.1152			M	**1229.4868**	615.2470	1212.4602	606.7338	1211.4762	606.2418	11
6	**659.3181**	330.1627	**642.2916**	321.6494			V	**1082.4514**	541.7293	1065.4248	533.2161	**1064.4408**	532.7240	10
7	756.3709	378.6891	739.3443	370.1758			P	**983.3830**	492.1951	966.3564	483.6819	**965.3724**	483.1898	9
8	**870.4138**	435.7105	853.3873	427.1973			N	**886.3302**	443.6687	869.3037	435.1555	868.3196	434.6635	8
9	927.4353	464.2213	910.4087	455.7080			G	**772.2873**	386.6473	**755.2607**	378.1340	754.2767	377.6420	7
10	1056.4779	528.7426	1039.4513	520.2293	1038.4673	519.7373	E	715.2658	358.1365	698.2393	349.6233	697.2553	349.1313	6
11	1127.5150	564.2611	1110.4884	555.7479	1109.5044	555.2559	A	**586.2232**	293.6153	569.1967	285.1020	568.2127	284.6100	5
12	1214.5470	607.7771	1197.5205	599.2639	1196.5365	598.7719	S	**515.1861**	258.0967	498.1596	249.5834	497.1755	249.0914	4
13	1328.5899	664.7986	**1311.5634**	656.2853	**1310.5794**	655.7933	N	428.1541	214.5807	411.1275	206.0674	410.1435	205.5754	3
14	1495.5883	748.2978	1478.5617	739.7845	1477.5777	739.2925	S	314.1112	157.5592	**297.0846**	149.0459	**296.1006**	148.5539	2
15							K	147.1128	74.0600	130.0863	65.5468			1

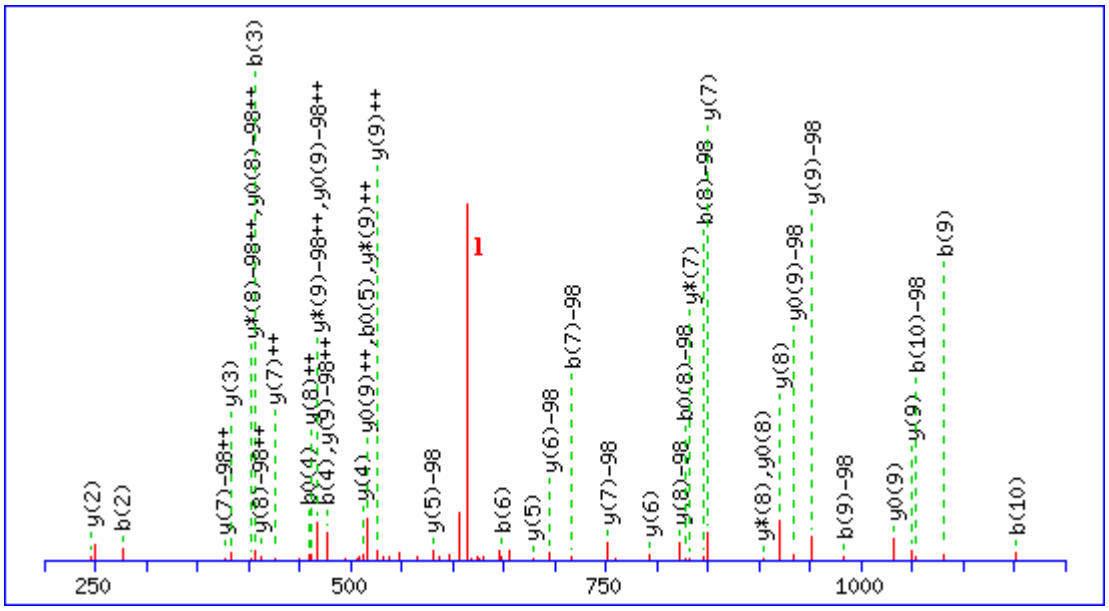
MS/MS Fragmentation of **VTPGAApSAAAGAK**



Monoisotopic mass of neutral peptide Mr(calc): 1150.5383
Variable modifications:
S7 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 72 **Expect:** 2.2e-06
Matches (Bold Red): 32/166 fragment ions using 28 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	100.0757	50.5415			V							13
2	**201.1234**	101.0653	183.1128	92.0600	T	1052.4772	**526.7422**	1035.4507	518.2290	1034.4666	517.7370	12
3	298.1761	149.5917	280.1656	140.5864	P	**951.4295**	**476.2184**	934.4030	467.7051	**933.4190**	467.2131	11
4	355.1976	178.1024	337.1870	169.0972	G	**854.3768**	**427.6920**	837.3502	419.1787	836.3662	418.6867	10
5	426.2347	213.6210	**408.2241**	204.6157	A	**797.3553**	399.1813	780.3288	390.6680	779.3447	390.1760	9
6	497.2718	249.1396	479.2613	240.1343	A	**726.3182**	363.6627	**709.2916**	355.1495	**708.3076**	**354.6575**	8
7	664.2702	332.6387	646.2596	323.6334	S	**655.2811**	328.1442	638.2545	319.6309	637.2705	319.1389	7
8	735.3073	368.1573	717.2967	359.1520	A	488.2827	244.6450	471.2562	236.1317			6
9	806.3444	403.6758	**788.3338**	394.6706	A	**417.2456**	209.1264	400.2191	**200.6132**			5
10	**877.3815**	439.1944	859.3710	430.1891	A	**346.2085**	**173.6079**	329.1819	165.0946			4
11	934.4030	467.7051	916.3924	458.6998	G	**275.1714**	138.0893	258.1448	129.5761			3
12	**1005.4401**	503.2237	**987.4295**	494.2184	A	218.1499	109.5786	**201.1234**	101.0653			2
13					K	147.1128	74.0600	130.0863	65.5468			1

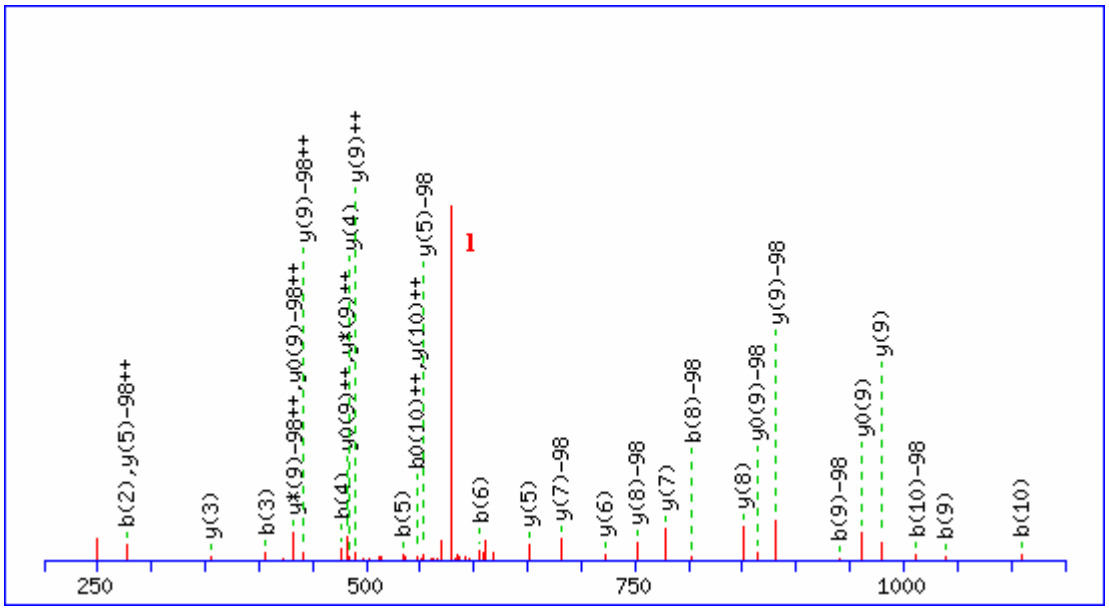
MS/MS Fragmentation of **YIEAGNpSEHAR**



Monoisotopic mass of neutral peptide Mr(calc): 1325.5401
Variable modifications:
S7 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 65 **Expect:** 1.5e-05
Matches (Bold Red): 43/160 fragment ions using 48 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	164.0706	82.5389					Y							11
2	**277.1547**	139.0810					I	1163.4841	582.2457	1146.4575	573.7324	1145.4735	573.2404	10
3	**406.1973**	203.6023			388.1867	194.5970	E	**1050.4000**	**525.7036**	1033.3735	**517.1904**	**1032.3895**	**516.6984**	9
4	**477.2344**	239.1208			**459.2238**	230.1155	A	**921.3574**	**461.1824**	**904.3309**	452.6691	**903.3469**	452.1771	8
5	534.2558	267.6316			**516.2453**	258.6263	G	**850.3203**	**425.6638**	**833.2938**	417.1505	832.3097	416.6585	7
6	**648.2988**	324.6530	631.2722	316.1397	630.2882	315.6477	N	**793.2989**	397.1531	776.2723	388.6398	775.2883	388.1478	6
7	815.2971	408.1522	798.2706	399.6389	797.2866	399.1469	S	**679.2559**	340.1316	662.2294	331.6183	661.2454	331.1263	5
8	944.3397	472.6735	927.3132	464.1602	926.3291	463.6682	E	**512.2576**	256.6324	495.2310	248.1191	494.2470	247.6271	4
9	**1081.3986**	541.2030	1064.3721	532.6897	1063.3881	532.1977	H	**383.2150**	192.1111	366.1884	183.5979			3
10	**1152.4357**	576.7215	1135.4092	568.2082	1134.4252	567.7162	A	**246.1561**	123.5817	229.1295	115.0684			2
11							R	175.1190	88.0631	158.0924	79.5498			1

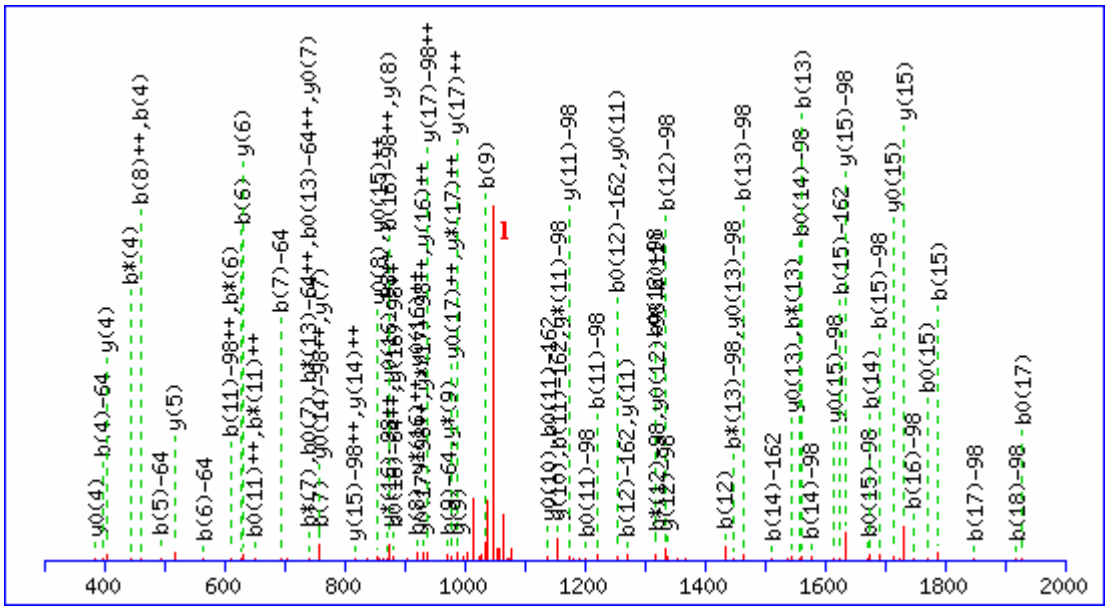
MS/MS Fragmentation of **YIEAGApSEHAK**



onoisotopic mass of neutral peptide Mr(calc): 1254.5281
Variable modifications:
S7 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 67 **Expect:** 7.2e-06
Matches (Bold Red): 32/142 fragment ions using 35 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	164.0706	82.5389			Y							11
2	**277.1547**	139.0810			I	1092.4721	**546.7397**	1075.4456	538.2264	1074.4616	537.7344	10
3	**406.1973**	203.6023	388.1867	194.5970	E	**979.3881**	**490.1977**	962.3615	**481.6844**	**961.3775**	**481.1924**	9
4	**477.2344**	239.1208	459.2238	230.1155	A	**850.3455**	425.6764	833.3189	417.1631	832.3349	416.6711	8
5	**534.2558**	267.6316	516.2453	258.6263	G	**779.3084**	390.1578	762.2818	381.6445	761.2978	381.1525	7
6	**605.2930**	303.1501	587.2824	294.1448	A	**722.2869**	361.6471	705.2603	353.1338	704.2763	352.6418	6
7	772.2913	386.6493	754.2807	377.6440	S	**651.2498**	326.1285	634.2232	317.6153	633.2392	317.1232	5
8	901.3339	451.1706	883.3233	442.1653	E	**484.2514**	242.6293	467.2249	234.1161	466.2409	233.6241	4
9	**1038.3928**	519.7000	1020.3822	510.6948	H	**355.2088**	178.1081	338.1823	169.5948			3
10	**1109.4299**	555.2186	1091.4194	**546.2133**	A	218.1499	109.5786	201.1234	101.0653			2
11					K	147.1128	74.0600	130.0863	65.5468			1

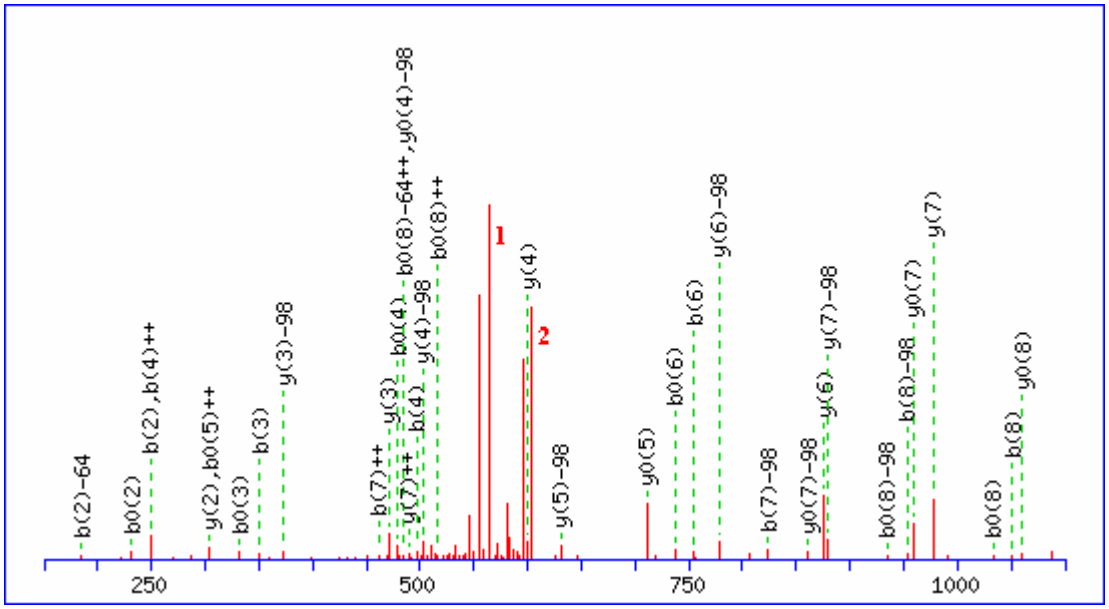
MS/MS Fragmentation of **AMNKPAEYDpSDDEIIGTAR**



Monoisotopic mass of neutral peptide Mr(calc): 2190.9140
Variable modifications:
M2 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
S10 : Phospho (S), with neutral losses 97.9769(shown in table), 0.0000
Ions Score: 81 **Expect:** 6.5e-07
Matches (Bold Red): 90/460 fragment ions using 74 most intense peaks

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	72.0444	36.5258					A							19
2	219.0798	110.0435					M	2022.9073	1011.9573	2005.8808	1003.4440	2004.8967	1002.9520	18
3	333.1227	167.0650	316.0962	158.5517			N	1875.8719	**938.4396**	1858.8454	**929.9263**	1857.8613	**929.4343**	17
4	**461.2177**	231.1125	**444.1911**	222.5992			K	1761.8290	**881.4181**	1744.8024	**872.9049**	1743.8184	**872.4128**	16
5	558.2704	279.6389	541.2439	271.1256			P	**1633.7340**	**817.3706**	1616.7075	808.8574	**1615.7234**	808.3654	15
6	**629.3076**	315.1574	**612.2810**	306.6441			A	1536.6812	768.8443	1519.6547	760.3310	1518.6707	**759.8390**	14
7	**758.3502**	379.6787	**741.3236**	371.1654	**740.3396**	370.6734	E	1465.6441	733.3257	1448.6176	724.8124	**1447.6336**	724.3204	13
8	**921.4135**	**461.2104**	904.3869	452.6971	903.4029	452.2051	Y	**1336.6015**	668.8044	1319.5750	660.2911	**1318.5910**	659.7991	12
9	**1036.4404**	518.7239	1019.4139	510.2106	1018.4299	509.7186	D	**1173.5382**	587.2727	**1156.5117**	578.7595	1155.5276	578.2675	11
10	1105.4619	553.2346	1088.4353	544.7213	1087.4513	544.2293	S	1058.5113	529.7593	1041.4847	521.2460	1040.5007	520.7540	10
11	**1220.4888**	**610.7481**	1203.4623	602.2348	**1202.4783**	601.7428	D	**989.4898**	495.2485	**972.4633**	486.7353	971.4793	486.2433	9
12	**1335.5158**	668.2615	**1318.4892**	659.7482	**1317.5052**	659.2562	D	**874.4629**	437.7351	857.4363	429.2218	**856.4523**	428.7298	8
13	**1464.5584**	732.7828	**1447.5318**	724.2695	1446.5478	723.7775	E	**759.4359**	380.2216	742.4094	371.7083	**741.4254**	371.2163	7
14	**1577.6424**	789.3249	1560.6159	780.8116	**1559.6319**	780.3196	I	**630.3933**	315.7003	613.3668	307.1870	612.3828	306.6950	6
15	**1690.7265**	845.8669	1673.6999	837.3536	**1672.7159**	836.8616	I	**517.3093**	259.1583	500.2827	250.6450	499.2987	250.1530	5
16	**1747.7480**	**874.3776**	1730.7214	865.8643	1729.7374	865.3723	G	**404.2252**	202.6162	387.1987	194.1030	**386.2146**	193.6110	4
17	**1848.7956**	924.9015	1831.7691	916.3882	1830.7851	915.8962	T	347.2037	174.1055	330.1772	165.5922	329.1932	165.1002	3
18	**1919.8327**	960.4200	1902.8062	951.9067	1901.8222	951.4147	A	246.1561	123.5817	229.1295	115.0684			2
19							R	175.1190	88.0631	158.0924	79.5498			1

MS/MS Fragmentation of **MTTFEEp*SER**



Monoisotopic mass of neutral peptide Mr(calc): 1224.4370
Variable modifications:
M1 : Oxidation (M), with neutral losses 0.0000(shown in table), 63.9983
S7 : Phospho (S), with neutral losses 0.0000(shown in table), 97.9769
Ions Score: 31 **Expect:** 0.014
Matches (Bold Red): 35/158 fragment ions using 50 most intense peaks

#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	148.0427	74.5250			M							9
2	**249.0904**	125.0488	**231.0798**	116.0435	T	1078.4089	539.7081	1061.3823	531.1948	**1060.3983**	530.7028	8
3	**350.1380**	175.5727	**332.1275**	166.5674	T	**977.3612**	**489.1842**	960.3346	480.6710	**959.3506**	480.1789	7
4	**497.2065**	**249.1069**	**479.1959**	240.1016	F	**876.3135**	438.6604	859.2869	430.1471	858.3029	429.6551	6
5	626.2490	313.6282	608.2385	**304.6229**	E	729.2451	365.1262	712.2185	356.6129	**711.2345**	356.1209	5
6	**755.2916**	378.1495	**737.2811**	369.1442	E	**600.2025**	300.6049	583.1759	292.0916	582.1919	291.5996	4
7	922.2900	**461.6486**	904.2794	452.6434	S	**471.1599**	236.0836	454.1333	227.5703	453.1493	227.0783	3
8	**1051.3326**	526.1699	**1033.3220**	**517.1646**	E	**304.1615**	152.5844	287.1350	144.0711	286.1510	143.5791	2
9					R	175.1190	88.0631	158.0924	79.5498			1